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The Yale Journal of Biology & Medicine

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ADDENDUM

To the paper entitled "A correlation of early radiation changes in lymphatic tissues with antitoxin-producing ability" by Williams, Stoner, and Hale in the June 1956 issue, page 615, there should be added the following statement: "This work was done under the auspices of the Atomic Energy Commission."

THE YALE JOURNAL
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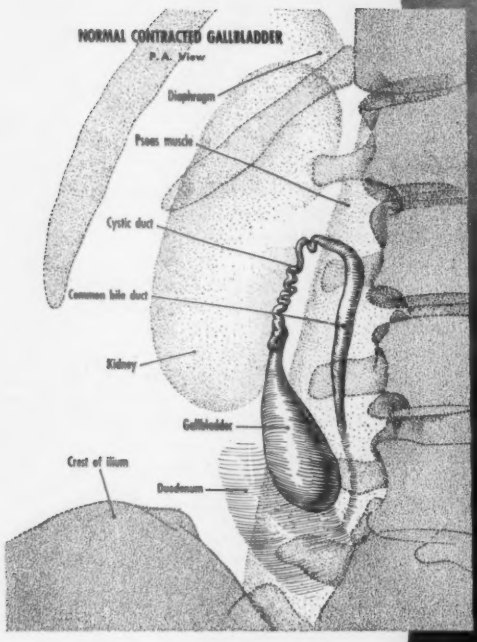

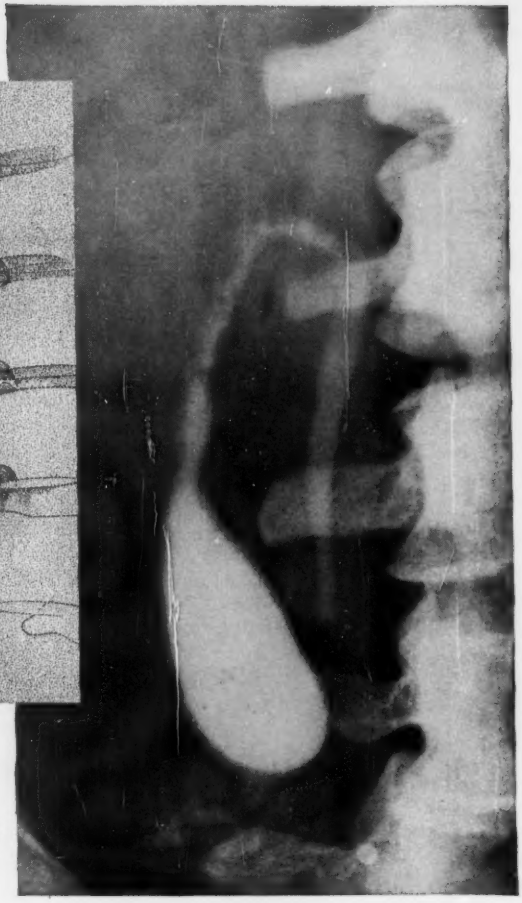

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FIRST THINGS FIRST IN MEDICINE†

MR. PRESIDENT, DEAN LIPPARD, ALUMNI, AND FRIENDS:

My address is a very personal homily, the substance of which befits the beliefs and experiences of the speaker rather more perhaps than it does the protocol of this occasion and the interests of this audience.

I would like to dedicate my remarks to the memory of five close friends and clinical colleagues—great physicians all—Jim Trask, Arthur Morse, Francis Blake, Sam Harvey, and Jack Peters.

After introductory comments and some words of caution regarding certain modern trends in medical education, I shall set down, as I see them, the items of prime importance in the education and character of those who aspire to high endeavor in medicine.

Scripture tells us that the Lord spoke out of the whirlwind to his servant, Job, saying:

"Who is this that darkeneth counsel by words without knowledge?"

Lest you chide me in similar fashion, I must present some credentials. I began participation in medicine forty-seven years ago and have thus observed the careers of a goodly number of students and house-officers in whom I have been keenly interested; there have been a few apparent failures but many successes far beyond the commonplace. Since my retirement from University activities in 1952, I have served as an official of the Committee on Education of the American Academy of Pediatrics. This is an organization of physicians certified by the American Board of Pediatrics.

In 1946-47 the Academy conducted a survey of child health services in the United States and Canada. As a part of this survey, there was an evaluation of pediatric education. This latter task was performed by a distinguished alumnus of the Yale School of Medicine and its Department of Pediatrics, now Dean of the Medical School of the University of Pennsylvania, Dr. John McKenny Mitchell. As one outgrowth of this appraisal of pediatric education, the Academy established a Committee on Education. Among its other activities this Committee sponsors group conferences of the

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* Professor Emeritus of Pediatrics.

† Presented at the meeting of Yale Alumni in Medicine, February 22, 1956.

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pediatric personnel of the several medical schools in the ten geographical regions into which the activities of the organization are divided—a program unique, I believe, in the educational circles of official medicine. For about three years I had the honor of attending many of these group meetings as a liaison officer for the committee.

Thus, as a member of medical school faculties at Johns Hopkins and Yale and as a participant in a number of recent conferences on medical pedagogy I have heard much on the education, training, and practice of physicians. Over the years I have had many contacts with young people in medicine and I am mindful of the admonition of our seventeenth century wise man, Sir Thomas Browne, who said: "Despise not the obliquities of younger ways, nor despair of better things whereof there is yet no prospect." However, I warn you of what an old-fashioned professor may now say, despite extensive exposure to modernism, by quoting with concurrence the remarks made by the violinist, Fritz Kreisler, on the occasion of his eightieth birthday: "There are things in life I don't like. It has become so modified that we lose the essentials to get the by-products. This is an age of velocity, and it has affected art also. Many things are being played too fast just to show dexterity and technique. Too many musicians have become tricksters. They take the heart out of music. When I was a young man you were a fine artist when your colleagues knew it; the millions did not have to. Artists were judged by what they could do, not by what they could draw at the box office. For all these reasons, I live in memories."

Following Mr. Kreisler's formula, what is it, first of all, that I do not like in modern medical education? It is bigness! Perhaps it is well to recall at this point, again with approval, the trenchant comments on this matter of size made by Prof. William James in 1899. In a letter to a friend he said: "I am against bigness and greatness in all its forms, and with the invisible molecular moral forces that work from individual to individual, stealing in through the crannies of the world like so many soft rootlets, or like the capillary oozing of water, and yet rending the hardest monuments of man's pride, if you give them time. The bigger the unit you deal with, the hollower, the more brutal, the more mendacious is the life displayed. So I am against all big organizations as such, national ones first and foremost; against all big successes and big results; and in favor of the eternal forces of truth which always work in the individual and immediately unsuccessful way, underdogs always, till history comes, after they are long dead, and puts them on the top."

Some medical schools have more than two hundred students per class. So long as state schools must admit applicants whose essential requirement is a home state university diploma, rest assured the limit has not been reached

in enrollment. Of course, bigger classes require enlarged facilities, less individual consideration, and permit less heart. I do not know how many physicians are needed to look after the health requirements of our increasing population but quality is the primary goal—and I do not think bigger and bigger medical centers are the means by which we shall get better and better doctors.

The second characteristic of modern medical education which I do not like is the great stress placed on the vital importance of curricular arrangement and curricular manipulation. I think courses and their arrangement are important but not of primary value since they are essentially the vehicles by which the teacher is able to demonstrate by example—that all-important characteristic of great teaching—how facts can ultimately be used in the healing art. The facts, for the most part, are in the books and journals available to everyone, as are the laboratories. But how they may be integrated, applied, and interpreted as respects the patient is a fair subject for the teacher's skill and wisdom. There is much emphasis on "scrambling," so called, of courses and combinations and re-arrangements so that sequences and relationships may be more logical. To my mind these academic acrobatics are largely mechanical and are of importance in relation to size of classes; they are in the same order of magnitude and quality as are, for example, audiovisual aids. Strong emphasis on this area of medical education is likely to weaken appreciation of the wise words of Comenius which Dr. Darrow often quoted: "Let the main objective be as follows: To seek and find a method of instruction by which the teachers may teach less, but learners may learn more."

One reason given for curricular changes is to get the students in contact with clinical medicine in the preclinical years when their enthusiasm for "doctoring" is at a peak. I am told that at an early session of one of these new-fashioned introductory or orientation courses given to first-year students one of them asked, "When are you going to tell us how to equip our offices?" That certainly is starting one's professional life before it has fairly begun! I do not minimize early enthusiasm for one's life work nor doubt that human interest is the strong motivation of many students to study medicine. But to capitalize on that motivation in the preclinical years at the expense of basic science—which in some measure must take place—is, in the long run, a disservice to clinical medicine. After all, if a student does not enjoy science and does not also have enduring interest in human beings, he should not continue the study of medicine.

In the third place I do not like the great emphasis on the necessity of organized programs designed to teach the practice of medicine—preceptorships, family or general practice clinics, home visiting, and the like. Medical

schools exist primarily to educate students in medical science; nowhere else is this obtainable. A four-year course is not a long time considering the magnitude of the material to be mastered. The facts of the sciences basic to medicine were never more extensive and complex than in our time. The more knowledge available, the more critical the responsibility to master and to use it—and there is no substitute for knowledge of basic science including psychology. The great advances in medicine are attributable in large measure to scientific discoveries; many of the various emotional and social aspects of medical practice, although often not expressed in modern terminology, have been recognized and practised for generations by the great clinicians to whom patients were never just "cases." The long training period of the modern internship and residency in addition to the clinical undergraduate years seems to me an especially appropriate time to emphasize these important aspects of the practice of medicine. With faculty goodwill and sincere interest a highly desirable but informal correlation of basic science and clinical study can be achieved all along the line.

Medical students are assumed to be ladies and gentlemen (and as such to have good manners including those appropriate at the bedside) and to be wise and compassionate human beings prepared for work on a graduate level at the time of admission to medical school. And it is to be assumed, also, that all teachers in medical schools exemplify by their performance the highest standards of professional and private excellence. If these assumptions are not in strict accord with the facts of life, I am quite sure they cannot be substantially altered by any courses or programs however elaborate.

Clearly, the most vital responsibility in the administration of a medical school is the choosing of students and teachers. As to students, character is important as well as high marks in college. As to teachers, humanitarian traits are important as well as scholarly bibliographies.

I do not wish to be understood as opposed to all efforts to relate medical science to medical practice; I simply desire to place first things first as I see them. I myself inaugurated a modest home visiting program on the newborn service here in 1939; and joint weekly rounds of the pediatric and social service staffs were conducted here for many years. These activities took place in postgraduate years where perhaps, as previously pointed out, they properly belong; the undergraduate clinical students, of course, saw these activities in operation.

Lastly, I do not like the excessive emphasis placed on the uniqueness of the recognition of the sociological and psychological components of illness as if good doctors ever ignored these matters. For if it is good clinical medicine, it seems to me that it is inclusive of whatever is meant by psycho-

somatic or comprehensive medicine; and the good clinician never neglects preventive measures and environmental relationships.

These ideas concerning the importance of emotional and sociological factors in illness are not new. They may be found expressed far back in medical history and are implicit in our language—hysteria, jaundiced eye, splenic, choleric, for example. I am indebted to Professor Bean of the State University of Iowa for the following illustration of psychosomatic medicine from the novel, *Humphry Clinker,* published by Tobias Smollett in 1771: "I find my spirits and my health affect each other reciprocally—that is to say, everything that discomposes my mind, produces a correspondent disorder in my body; and my bodily complaints are remarkably mitigated by those considerations that dissipate the clouds of mental chagrin. The imprisonment of Clinker brought on those symptoms which I mentioned in my last [letter], and now they are vanished at his discharge. It must be owned, indeed, I took some of the tincture of ginseng, prepared according to your prescription, and found it exceedingly grateful to the stomach; but the pain and sickness continued to return, after short intervals, till the anxiety of my mind was entirely removed, and then I found myself perfectly at ease."

Again, lest I be misunderstood and be considered as opposed to psychiatry and social science, may I remind you that one of the first assignments of a child psychiatrist to a pediatric department took place here in 1930 when Dr. Marian C. Putnam began working with our child patients in outpatient and in-patient services. And, further, one of the earliest contemporary Rooming-In projects was inaugurated here by Dr. Edith B. Jackson on the newborn service in 1946. This project has an excellent record in nurturing physicians later specializing in psychiatry and child guidance.

These facts and trends—and others which I have not mentioned—are said to indicate that medical education is in transition from disease orientation to patient and community orientation. Rather should one say, I think, that medical practice, not strictly medical education, is shifting its focus somewhat. I am not sure how basic all this is, but I do not oppose it and in the main approve, provided it is regarded, in large measure, as experimental and exploratory. I am simply taking this opportunity to de-emphasize—gently, I trust—what I consider to be relatively minor and to stress now matters in addition to basic scientific knowledge, which I personally place foremost for medicine of top quality—matters which are never in transition and never dated.

The first to be mentioned is scientific inquiry. This may seem an old story to alumni of this school; but does the spirit of scientific inquiry play a dominate rôle in the training and practice of the majority of the more than

200,000 physicians in this country? If it did, would they prefer dosing with antibiotics to accurate diagnosis and rational therapy? Would they so often succumb to the "malignant itch to cut"? Would they so frequently cease to be students as soon as state board licenses and board certificates are received? And would they depend principally upon the advertisements of pharmaceutical houses, excellent as many of them are, for reviews of the scientific literature?

The submission of a thesis has been required in this medical school for many years. Our registrar, Miss Belehrad, found in the catalogue of the Medical Institution of Yale College for 1845 that even the "form of paper" on which it was to be written as well as the dissertation itself was prescribed. A critical review and appraisal of the merits and educational philosophy of these essays and reports of investigations would seem a proper subject for future pedagogical exploration. I, a clinician, believe experience in a research project at the medical undergraduate level is of potentially greater importance than learning *at that stage* how to appraise accurately and sympathetically community and personal health situations. Of course, small classes lend themselves effectively to activities of this type and herein is one of the many counts against "bigness" in medical education. One reason research is so important at that time is that only in the setting of formal medical education are opportunities and facilities for exact and supervised observation and study of a special problem available to most students. In contrast, some experience with various social and psychological processes is bound to come sooner or later to every physician. I am not intending by these remarks to emphasize relative importance but rather sequence, availability, and opportunity. Dean Lippard stresses the point that as a result of this experience in research (the thesis) "a few students will have kindled the spark of curiosity which will lead them to a career in academic medicine and many others will become better practitioners." Experience in research, particularly in the evaluation of data, is the most satisfactory and concentrated means of which I have knowledge by which the student may develop criticalness and maturity of judgment—assets of supreme value to the good physician.

From personal knowledge I know something of the close intellectual comradeship and warm friendship which the thesis experience often develops between student and faculty adviser. These relationships have afforded most rewarding and enduring stimuli to both participants. Although this part of the Yale medical discipline may be a waste of time for certain students and for certain teachers, the experience in many other apparently unpropitious instances brings out valuable, latent talents not elicited by other studious pursuits.

Yale seems to be the only medical school which requires a dissertation before awarding the M.D. degree; but I, a clinician, offer no apology for my strong support of this particular method of developing research and critical interests in students. But certainly it should be very clear that a graduation thesis is not essential for the investigative orientation of an institution. This spirit after all is really dependent on students and teachers, not on mechanism, although I think this helps, as does tradition. The climate of the school must be permeated with the spirit of curiosity, with scientific imagination and skill. Herein lie the great contributions which teachers can make.

One should further point out that the thesis project promotes library experience which in conjunction with the leisurely study and preparation afforded by a liberally elective curriculum encourages scholarly endeavor, valuable alike to future academician and private practitioner.

The other high qualifications which I consider essential to superior achievement in medicine are integrity of character and dedicated personal service; an educated mind without a dedicated spirit is not enough. May I briefly illustrate what I mean by two examples—one from eighteenth century Britain and the other from contemporary America.

The first example is Dr. George Armstrong. My good friend, Dr. Ernest Caulfield—our distinguished historian of pediatrics—has conveyed to me some of his interest in Armstrong who wrote a little book, first published in London in 1767, entitled *An Essay on the Diseases Most Fatal to Infants.* The excellence of the clinical medicine described in this book merits for Armstrong the sobriquet, "Father of Modern Pediatrics." So much for the achievements of the educated mind!

In 1769, two years later, Armstrong founded in London the Dispensary for the Infant Poor, "the first and only charity of the kind that has ever been established." There is no doubt that to the patients who were brought to this dispensary, Armstrong gave all the skill, knowledge, and devotion of which he was possessed, and the institution flourished under his almost single-handed efforts. This meant neglect of the remunerative part of his practice by which he earned his livelihood. The result, a debtors' prison for both Armstrong and his wife—and immortality in the history of Great Medicine. This was the reward of his dedicated spirit in eighteenth century Britain.

The second example. Some years ago an alumnus of this school and its department of pediatrics became chief resident in a large university hospital not too far distant. In the course of time I received a letter from a friend who is chief of the pediatric service in that hospital. I sent the letter to the

mother of the young doctor so I cannot quote it exactly but the message it carried was as follows:

I thought you would like to have me tell you of an experience I had the other night with Dr. Blank. I was called to the hospital at midnight by another member of the house staff to see a private patient. As I passed the public ward I saw Dr. Blank working at the bedside of a sick baby. I happened to know it was his night off duty and I chastized him gently for staying around. I thought you would like to know his comment—where I come from you do not leave the ward when you are needed to care for a sick patient.

That is just one example, of many I might cite, of contemporary medical America's dedicated spirit in personal service—something which counts not the cost of duty and knows not the frustrations of the time clock!—a character trait which, of course, is deeply rooted in early life but may be brought to full flower in a propitious environment—one of the great opportunities and responsibilities of the teachers and students of a medical center to create and maintain.

In summary, then, I have been critical of certain currently popular developments in medical teaching. Let me leave you with the thought that I am not criticising these developments in so far as they are experimental and exploratory but only as they seem in some minds to be acceptable in lieu of and at the expense of basic knowledge, scientific inquiry, and dedicated personal service.

But a quotation from Dr. Cushing expresses my concluding emotions:

". . . the more years I spend in the business (of medical education), the greater diffidence I feel in expressing any view whatsoever. Such as they are, those which I now think I hold are almost certain to be regarded as old fashioned if not reactionary, which is embarrassing."

L. L. WATERS*†

Department of Pathology, Yale University School of Medicine

STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE

The Effect of Intravenous Egg-Yolk Emulsions on Inflammatory Lesions of the Aorta and Coronary Arteries of Dogs‡

Long study of human post-mortem material and of the arterial disease in rabbits that follows cholesterol feeding has not yet resulted in an understanding of the pathogenesis of arteriosclerosis. Although the focal distribution of the lesions in man and the partition of their contained lipids suggest interactions of local and hemic factors, it has proven difficult to determine either the nature of these factors or to clarify their interrelationships. The report that follows describes an experimental procedure for the investigation of certain aspects of this subject. The technique utilized allows examination of the effects of altered blood lipids on the morphological sequences of experimentally produced inflammatory lesions of the aorta and coronary arteries of dogs.

Anitschow[1] early spoke of a "combination theory" of the pathogenesis of arteriosclerosis and was able to demonstrate the deposit of lipids in the aortas of rabbits fed cholesterol and given epinephrine. The fact that it was quickly shown that the feeding of cholesterol alone resulted in fatty arterial lesions undoubtedly diverted attention from possible local factors involved in their establishment. More recently, Schlichter *et al.*[17] cauterized the aortas of cholesterol-fed dogs and reported fatty deposits at the site of the lesions in some instances. Kelly and Taylor and their associates[5, 9, 20] have carried out similar experiments in rabbits and monkeys. The abdominal aorta was injured by freezing during the course of a high-fat, high cholesterol regimen and fatty transformations of the lesions so produced have been described and the subsequent processes of repair studied. Acute arterial lesions have also been produced in rabbits by sensitizing procedures during hypercholesterolemia.[18] Acceleration of lipid deposit in the arterial lesions resulted.

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* Associate Professor of Pathology.

† With the technical assistance of Edward Iannucci, Peter Integlia, and Helen Cavallero.

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In this laboratory, the localization of particles from the blood, such as india ink,[11] methyl cellulose,[20] or fat,[4, 12] has been observed to occur selectively in areas of arterial inflammation. The deposit of lipids in sites of acute arterial injury in vitamin D poisoning in dogs has also been described,[10] and more recently it was found that the hyperlipemia resulting from intravenous administration of detergents in dogs was associated with fatty foam-cellular transformation of inflammatory lesions of the coronary arteries.[3] The present experiments, begun in 1953,[22] are reported in some detail because they have yielded information of interest.

The general plan has been to produce selective injury of the coronary arteries of dogs by the intravenous administration of allylamine.[21] Concomitantly, alteration in the pattern of blood lipids was effected by the intravenous infusion of varying quantities of saline emulsions of egg-yolk. This method for artificially changing the blood lipids was suggested by the early observations of Bloor.[5] Following these procedures, numerous lesions of the coronary arteries are present within a few hours in 80-90 per cent of the animals and have progressed to partial or complete obliteration of individual coronary arteries within a two-week period.

MATERIALS AND METHODS

Healthy, adult mongrel dogs maintained on low-fat, stock laboratory diets were utilized. An occasional young or old animal was intentionally included, but the results in these individuals did not differ from those of the group. The observations described are based on a study of over 100 animals.

The method for the production of acute lesions of the coronary arteries in dogs by allylamine, and a description of these lesions has been published elsewhere.[21] In brief, a 1 per cent aqueous solution of allylamine (Eastman Kodak Co.) was freshly prepared. This was neutralized to pH 7.4 with normal HCl and was injected intravenously. Dogs were given a course of 15-20 mgm.base/kg. on each of the first three days of the experiment or on days 1, 3, and 4, or 1, 2, and 4, depending on the condition of the animal.

Emulsions of egg-yolk were prepared as follows: To 500 ml. of sterile, fresh, washed yolks, 700 ml. of sterile 0.9 per cent saline was added. The mixture was stirred and filtered through several layers of sterile gauze. The lipid and calcium content of an average lot of the emulsion follows:

Total cholesterol	503.0 mgm.%
Free cholesterol	473.3 mgm.%
Fatty acids	388.0 mEq./l.
Lipid phosphorus	138.0 mgm.%
Calcium	32.0 mgm.%

The contained cholesterol was almost all in the free form. The lipid-rich yolk particles were nearly uniform in size, as in native yolk, and measured approximately 1 μ in diameter.

The majority of animals received varying amounts of this emulsion intravenously beginning usually on the day of the first allylamine injection and thereafter daily on every second or third day for different periods ranging from 3 to 21 days. The minimal lethal dose of egg-yolk for a single intravenous injection was found to be above 30 ml./kg. While standard dose for the rapid production of fatty lesions was 20 ml./kg. and most of the changes described followed use of this amount, regimen was varied according to the purpose of the experiment.

Lipids and calcium in the plasmas of recipient animals were determined by the following methods: cholesterol and cholesterol esters—modified Schoenheimer-Sperry;[19] lipid phosphorus—a modified Youngburg procedure;[6] fatty acids—a modified Stoddard and Drury technique;[13] calcium—the method of Roe and Kahn.[16] Blood non-protein nitrogen was estimated by the usual micro-Kjeldahl digestion and steam distillation of the ammonia formed.

Complete autopsies were performed at the termination of each experiment and histological study carried out. The tissues were fixed in neutral formalin and frozen or paraffin sections prepared. Frozen sections were stained with sudan IV, and with the aid of a modified Schultz reaction. Paraffin sections were stained by Masson's method, hematoxylin and eosin, or Verhoeff's technique for elastic tissue.

EXPERIMENTAL

Effects of intravenous injection of egg-yolk emulsions in dogs

Unanesthetized, normal, or allylamine-injected dogs frequently exhibited dyspnea, vomiting, and increase in cardiac rate during and following the injection of large quantities of saline emulsions of egg-yolk. These signs were transient and were rarely severe. There was no sustained change in mean femoral arterial pressure. Reactions of the type described became less marked after the initial injection of the emulsion, and the majority of the animals remained in good condition. Occasionally, with successive large injections, an animal succumbed apparently from circulatory overload. If the egg emulsion was not fresh, its toxicity increased greatly.

Of particular interest were the effects of the injections on the blood. The plasma immediately following infusion was regularly yellow and lactescent and contained innumerable particles of the emulsion in suspension. This turbidity cleared progressively over a 24- to 72-hour period. At the end of this time, the plasma was opalescent with a greatly reduced number of suspended particles. It was distinctly yellow in contrast to the usual appearance of the plasmas of normal dogs on low fat diets. Only rarely could this appearance be explained by prior hemolysis or contained bilirubin. Even after repeated injections of egg-yolk there was no increase in plasma non-protein nitrogen. The blood lipids were profoundly altered. By a dilution-precipitation technique,[7] it was possible to demonstrate large quantities of dissolved lipovitellin at times when the plasma was practically particle-free. Fractionation of the plasma revealed an enormous increase in all lipids. In

Table 1 are summarized the pre- and post-injection values for a dog's plasma lipids with 20 ml./kg. egg-yolk emulsion intravenously.

Since egg-yolk contains practically no ester cholesterol, it is interesting to note that the plasma ester cholesterol was elevated appreciably at 48 hours and that total cholesterol fell more slowly than free cholesterol or the other lipid fractions. Table 2 indicates the changes that occurred in dogs' plasma cholesterol and calcium following repeated daily injections of intravenous egg-yolk.

It is noteworthy that in many animals a rough leveling off of the greatly elevated values occurred after a few days (Dog 1103), whereas in others

TABLE 1. EFFECT OF A SINGLE INTRAVENOUS INJECTION OF EGG-YOLK EMULSION (20 ML./KG.) ON THE PLASMA LIPIDS OF A FASTING DOG

Dog #1189 Time	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Lipid phosphorus mg.%*	*Total fatty acids mEq./l.*
Before injection	104	31.4	10.6	12.2
5 min. after injection	289	219	50.2	147.7
24 hrs.	250	175	41.0	129.3
48 hrs.	245	70	17.0	25.4
72 hrs.	183	45	11.0	15.8
96 hrs.	176	38	11.2	14.4

(Dog 1104), the values progressively increased. In the latter group, the animals appeared to be in circulatory distress as evidenced by low blood pressure and a shock-like clinical state. They succumbed if the injections were continued. During the course of injections with egg-yolk, plasma calcium was elevated. Besides containing calcium, yolk is a rich source of vitamin D. The occasional calcification occurring in the arterial lesions may be associated with these factors. Serum non-protein nitrogen values were not altered.

Morphological changes in the cardiovascular system of dogs receiving egg-yolk emulsions

Aorta. In the normal adult dog, the intima of the aorta is thin and consists of the endothelium with a few connective tissue fibers lying directly upon the internal elastic membrane. The aortic media is thick, with regularly spaced elastic and muscle fibers invested by a fibrous connective tissue complex. Numerous vasa vasorum are present in the adventitia and in ordinary preparations may be followed into the outer media.

Within a few minutes after the intravenous injection of a quantity of egg-yolk, diffuse sudanophilic material could be demonstrated microscopically in the ascending aorta beneath the endothelium and in the fibrous connective tissue spaces of the inner media. Within two to three hours, the inner third of the media was stained. After 24 hours, yellow discoloration of

TABLE 2. THE EFFECT OF DAILY INTRAVENOUS INJECTIONS (20 ML./KG.) OF EGG-YOLK EMULSION ON THE PLASMA LIPIDS AND CALCIUM OF FASTING DOGS

Dog #1103 Time	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Lipid phosphorus mg.%*	*Fatty acids mEq./l.*	*Calcium mg./%*
Control	75	30	9.0	6.2	12.0
Day 1	358	190	33.0	84.0	14.0
Day 2	550	355	29.5	144.0	14.8
Day 3	550	387	53.0	142.0	18.0
Day 4	750	270	50.0	153.0	28.0
Day 5	638	475	43.0	145.0	22.0
Day 6	800	520	43.0	185.0	30.0
Day 7	850	433	58.0	159.0	24.4
Dog #1104					
Control	158	40	14.5	11.6	12.8
Day 1	500	295	46.0	115.0	15.6
Day 2	750	455	80.0	156.0	19.5
Day 3	825	575	58.0	203.0	15.2
Day 4	900	675	65.0	216.0	18.6
Day 5	1025	725	85.0	226.0	17.6

the ascending aorta was discernible grossly and the inner portion of the vessel wall was strongly sudanophilic (Fig. 1). It became apparent, when this process was followed serially, that infiltration of lipid occurred from the intimal aspect of the vessel. In the fibrous connective tissue of the outer media, halos of sudanophilia surrounded the vasa vasorum. These stained areas were distinct from the lipid-stained inner medial layers and intima. In paraffin sections of the aorta, stained with Masson's technique or by hematoxylin-eosin, little change was to be seen in the first 24-48 hours except for subendothelial and medial edema. Necrosis was not evident.

After the first 48-72 hours following a single large injection, the sudanophilia of the inner media began to fade, but droplets of lipid accumulated in mononuclear phagocytes both beneath the endothelium and in the media. The subendothelial space was broadened and in it there was a

progressive accumulation of lipid in droplet form. Paraffin sections revealed subendothelial proliferation of connective tissue cells and the accumulation of fat-filled phagocytes. The impression was gained that the latter derived both from fibroblasts and from transformation of once-free mononuclear cells.

With repeated large injections of egg-yolk alone and with the combination of allylamine injections, the proliferative and fatty intimal changes in the aorta became much more conspicuous (Fig. 2). After ten days or two weeks on such a regimen, in the ascending aorta and at the junctions of major and minor branches, distinct, foamy subendothelial proliferations were present (Figs. 3 and 4). Very little diffuse or extra-cellular lipid was found in these lesions. Similar changes, often equally severe, were frequently found in the first portion of the pulmonary artery.

Following cessation of egg-yolk injections, the aortic intimal lesions over a period of three to six weeks gradually lost their content of lipid and progressed to a dense, flat, intimal scar, extending over an area corresponding to the original lesion. No permanent changes in the media or adventitia have been observed.

Morphological changes in coronary arteries of dogs following injection of egg-yolk or of allylamine alone.

The repeated intravenous injection of 20 ml./kg. of egg-yolk emulsion resulted in no arterial lesions of coronary arteries of *normal* dogs. This held true even when the plasma cholesterol had been maintained for a week or more in the range 500-1000 mg. per cent. With preceding or concomitant injections of allylamine, fatty coronary artery lesions occurred at once and were widespread, stressing the importance of injury for the localization of lipids in arteries.

To describe adequately the development of the fatty arterial lesions that follow allylamine and egg-yolk injection, it is necessary first to summarize the changes in the coronary arteries that follow the administration of allylamine alone. Within three to six hours after an injection of 15-20 mg./kg. of the neutralized base, areas of medial edema appear in segments of the coronary arteries. Necrosis of the media ensues within 24-48 hours and at the end of five days a fully developed segmental panarteritis is present (Fig. 5). Besides the medial necrosis and edema, there is a predominantly mononuclear, periadventitial cellular exudate, and beginning proliferation of fibroblasts in the adventitia and media. The intima is often the seat of a productive endarteritis. In many instances, the endothelium remains intact, but is raised from the internal elastic membrane by the accumulation of underlying fluid. In the subendothelial space so created,

large and small mononuclear cells accumulate along with various quantities of fibrin-like material. Gradually, as reparative phenomena become more prominent, necrotic medial muscle is replaced by hyalinized scar; adventitial exudate by fibroblasts. The mononuclear cells in the subendothelial space are either transformed into or are replaced by young collagen-forming cells, and the space is converted into a fibrous intimal plaque or scar, which partially occludes the vessel (Fig. 6). Occlusive thrombi are only rarely associated with the lesions, the endothelium usually remaining intact. When it is denuded, an interesting sequence develops. Strands of fibrin bridge across arcs of the lumen of the affected vessel. Endothelial cells quickly grow out along these strands so that a subendothelial space is reformed. Mononuclear cells accumulate and the new space is organized into a fibrous intimal plaque. It should be emphasized that the changes in even a large vessel, from the initial medial edema to a stable state of intimal, medial, and adventitial scar, occupy a period of about ten days to three weeks. Smaller vessels complete the process of inflammation and repair in even less time.

Allylamine lesions in control animals on standard low fat laboratory diets have consistently exhibited little or no stainable lipid. Occasionally during the stage of medial necrosis a light sudanophilia of the most affected medial areas can be demonstrated, but beyond this the basic, control allylamine lesion is sudan-negative.

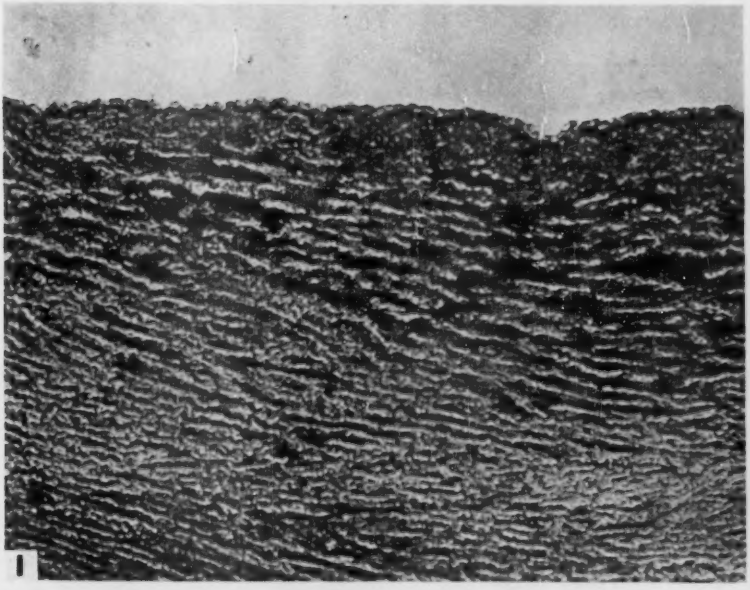
The effects of intravenous injection of egg-yolk emulsion on the basic allylamine lesion of coronary arteries

Within minutes after the intravenous injection of egg-yolk emulsions, the acute lesions of the coronary arteries of dogs that follow allylamine administration began to stain positively with sudan dyes. The staining was sharply localized to the areas of acute inflammation, was diffuse, and did not differ appreciably from the sudanophilia of the animal's lipid-rich plasma. Within 12-24 hours, the accumulation of lipids was massive, yet was selectively localized to areas of injury. Droplets of sudanophilic material might now be observed in areas that earlier stained diffusely. These droplets increased in number and were extracellular. Accumulations of lipid as described were present in early lesions in the necrotic media. Lipid also appeared in large quantities beneath the elevated endothelium; in other words, wherever fluid exudate was present in the inflammatory focus. Hand in hand with the disappearance of edema from the lesions and with the occurrence of the proliferative stages of repair, the lipid was progressively incorporated into mononuclear phagocytes and into young fibrocytes. This incorporation was massive, converting the organizing subendothelial space, or the new intimal

compartments formed by endothelization of fibrin strands, into fatty foam-cellular granulomas (Figs. 7, 8, 9, 10, 11, 12, 13). Evidence of the subsiding inflammatory reaction, in the form of residual fibrinoid masses, inflammatory leucocytes, and red blood cells, was still present in the early stages of this process, so that the histological appearance simulated that of an inflammatory xanthoma of the artery wall. The foam-cell granuloma might be confined to the proliferated intima or might involve the media to some degree as well. The early stages of the fatty lesion did not stain for cholesterol, but after ten days to two weeks the Schultz test was often positive.

After ten days to two weeks, little evidence of the previous exudative inflammatory reaction remained. The foamy intimal plaque was fully developed (Figs. 14, 15, 16). The component foam cells were moderate in size, with finely granular cytoplasm and small dense nuclei. If the plaque was large, it received a newly formed capillary blood supply from the adventital vasa vasorum. These small vessels traversed the media to supply the plaque. Other blood-vascular channels of various size might be present within the newly formed intimal granuloma. These suggested the "recanalizations" of organized thrombi. Consecutive sections have shown them to be sinusoidal-like ramifications of the lumen of the parent vessel associated with irregularities in the surface of the intimal plaque.

Directly following the development of the xanthomatous stage, in the second or third week, "regressive" changes set in. These refer not to disappearance of the lesion, which did not occur, but to a progressive loss of the contained lipids and to a progression of the connective tissue cells of the granuloma to scar. This process occupied two to three weeks. The fatty, foam cells became smaller with more sharply defined cell borders. Their cytoplasmic content appeared dense and darker and gradually the contained lipid disappeared. At the same time, the connective tissue fibers of the plaque became more numerous, resulting in a dense, fine-fibered collagenous scar (Figs. 17, 18). Small blood vessels have persisted for months within such connective tissue plaques. Loss of lipid and condensation of the connective tissue of the lesions to scar took place even if injections of allylamine and of massive quantities of egg-yolk lipid were continued. Apparently, a particular segment of the vessel took in lipid-rich plasma over a limited time, probably only during the early stage of inflammation when permeability was increased. Certainly after a period associated with the disappearance of edema from the lesions, the lesions of other animals in the series at the same stage of the experiment could not be made to localize newly administered lipid. However, after a time interval of two to three months the vessels could be re-injured, as will be described.



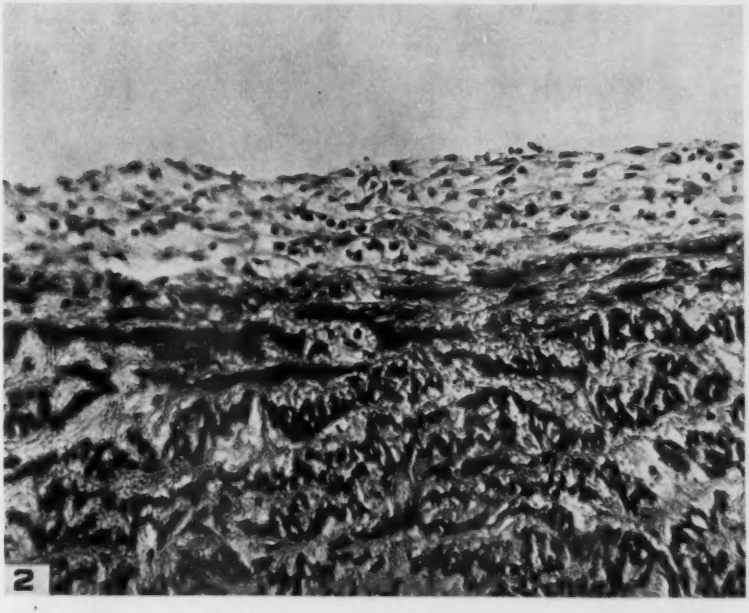


FIG. 1. Normal dog. Sudan IV stain. Ascending aorta twenty-four hours after large intravenous injection of egg-yolk. Dark areas represent lipid. Lumen of vessel above. x140.

FIG. 2. Normal dog. Fatty, foam-cellular lesion of ascending aorta four days after six daily intravenous injections of egg-yolk. x300.

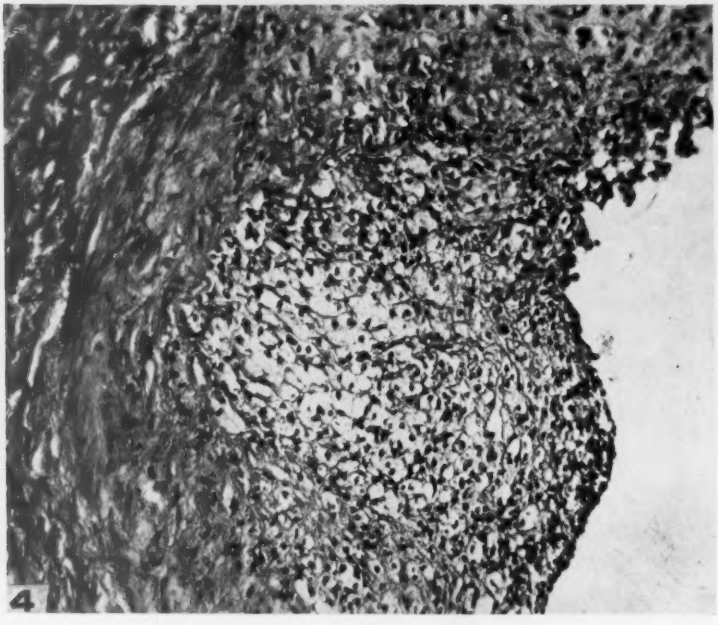


FIG. 3. Dog 1016. Thoracic aorta at exit of intercostal artery. Foam-cell granuloma. Three injections of allylamine, six of egg-yolk. Eleven days. x60.

FIG. 4. Dog 1016. Higher power of foam-cell granuloma in Figure 3. The lipid is intracellular. x320.

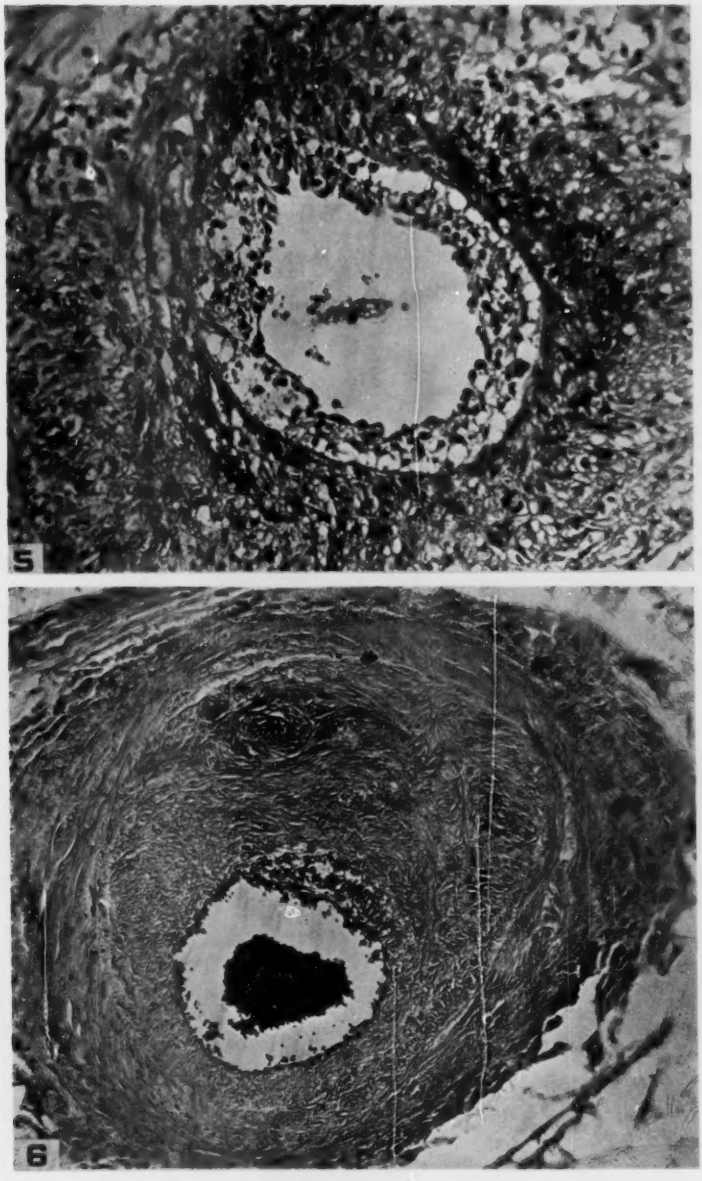
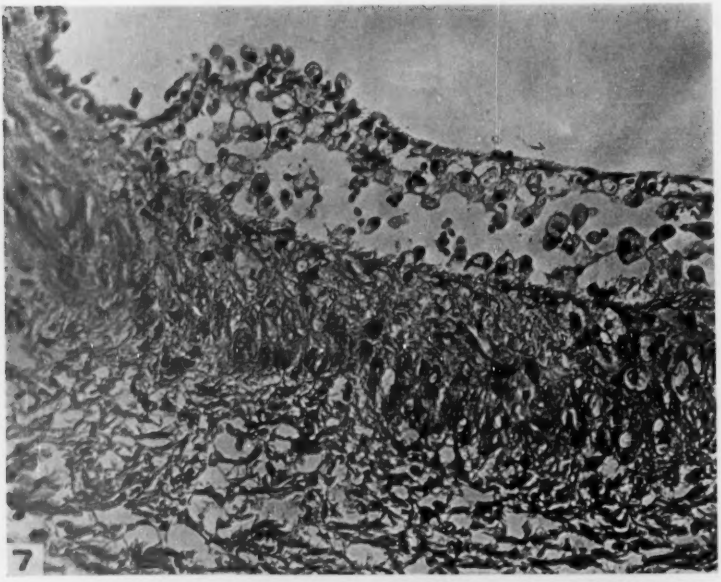


FIG. 5. Dog 801. Coronary artery two days after a course of three allylamine injections. Segmental panarteritis with proliferative intimal component. Lesion sudan negative. x250.

FIG. 6. Dog's coronary artery six weeks after three injections of allylamine. Fibrous intimal scar, supplied by small, newly-formed vascular channels. Lesion sudan negative. x200.



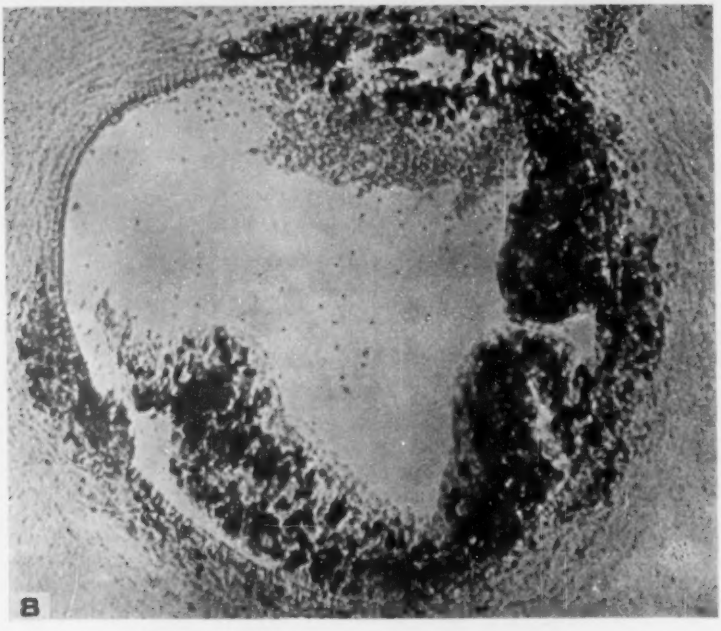
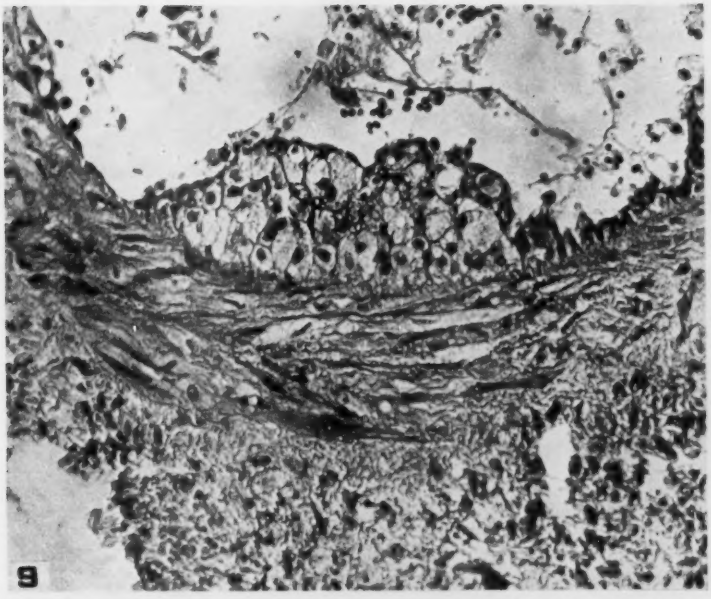


FIG. 7. Dog 1085. Coronary artery ten days after three allylamine and eight egg-yolk injections. The endothelium is raised and lipophages are accumulating beneath it. x380.

FIG. 8. Dog 1085. Sudan IV stain of coronary artery ten days after a course of allylamine and egg-yolk injections. The dark masses represent lipid in the intima and injured medial segments. There is no lipid in the uninjured parts of the vessel. x200.



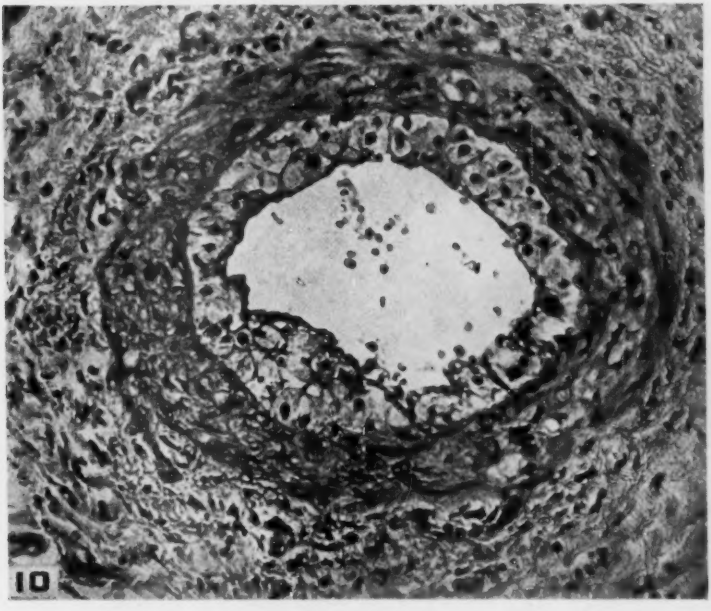


FIG. 9. Dog 1015. Coronary artery eleven days after allylamine and repeated egg-yolk injections. The space beneath the elevated endothelium is filled with lipophages. x400.

FIG. 10. Dog 1075. Coronary artery. Another example of subendothelial foam-cell granuloma eleven days after three allylamine and seven egg-yolk injections. x375.

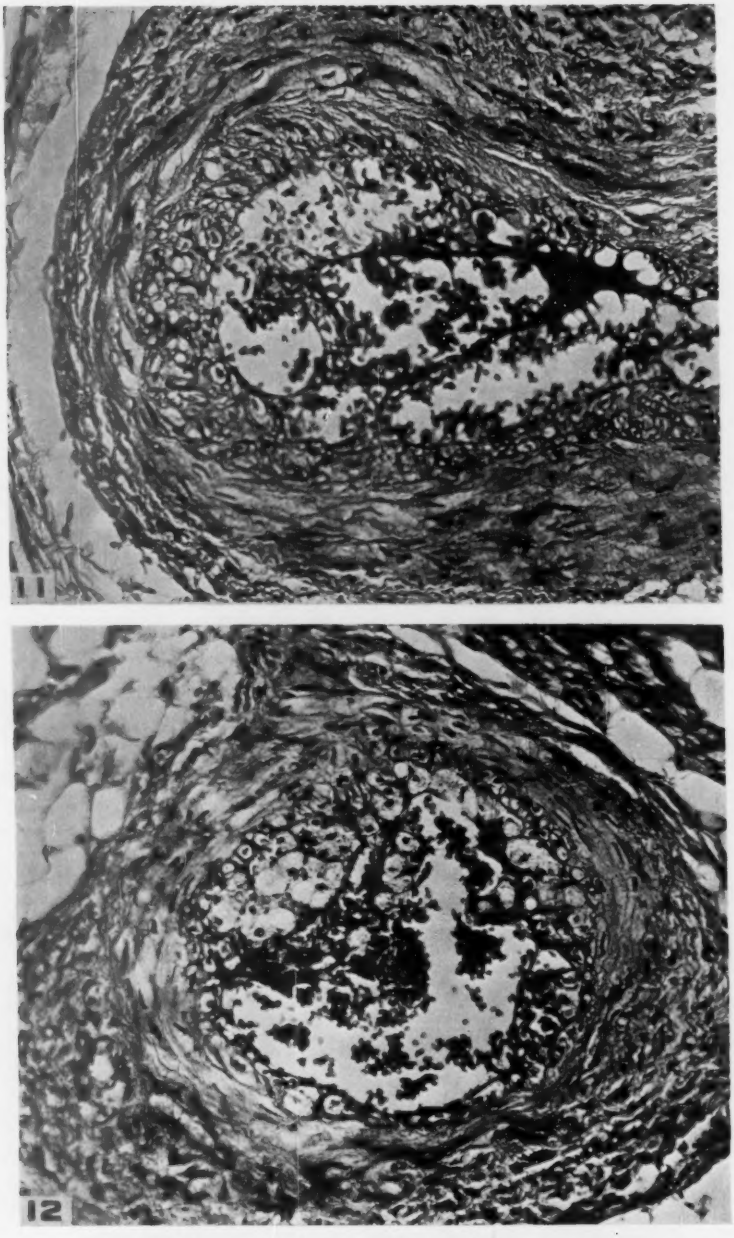
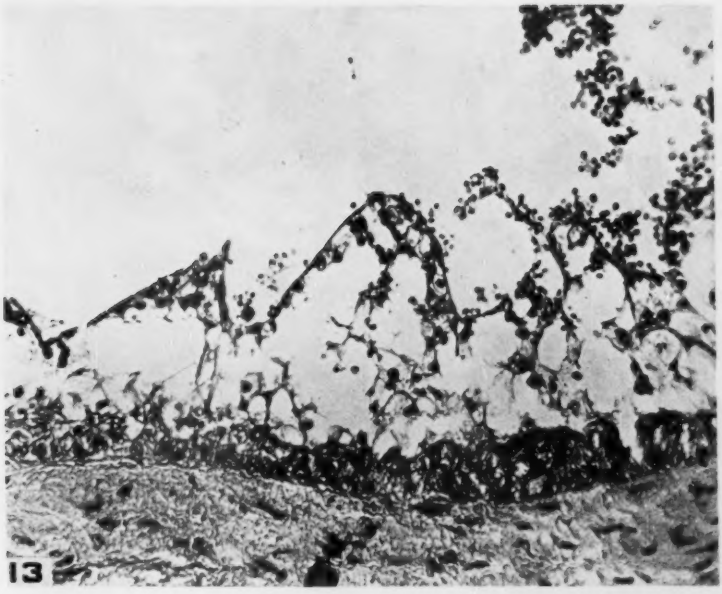


FIG. 11. Dog 1076. Coronary artery. Combined allylamine, egg-yolk administration. Twelve days. Formation of new subendothelial space by organization of fibrin bridges. Fibrin appears as dark lace work surrounding a central channel containing red blood cells. x300.

FIG. 12. Dog 1076. Coronary artery. A further extension of the process in Figure 11. Accumulation of lipophages beneath the endothelium. x300.



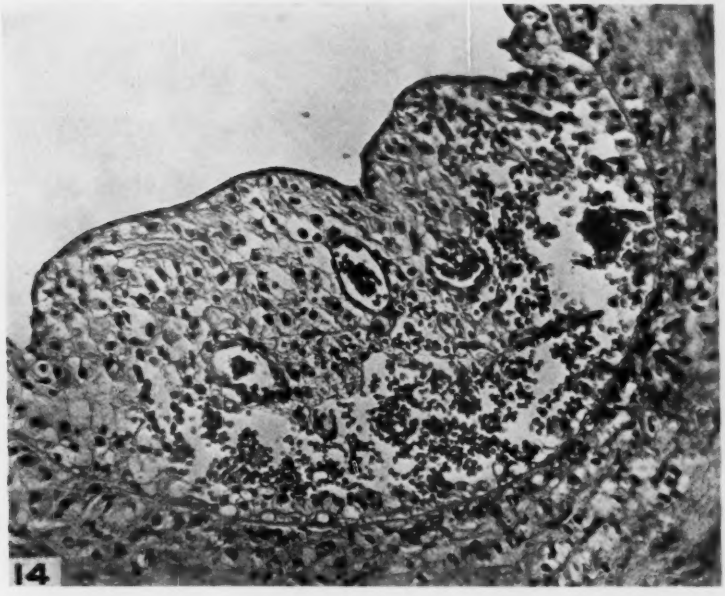
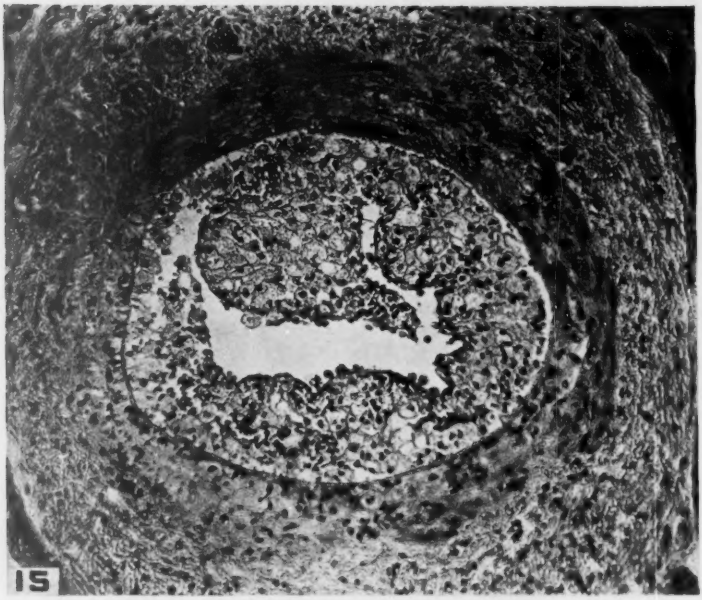


FIG. 13. Dog 1084. Coronary artery. Combined allylamine and egg-yolk injury, nine days. The newly formed endothelium is distinct. Fibrin has largely disappeared and subendothelial monocytes are being transformed into lipophages. x375.

FIG. 14. Dog 1085. Coronary artery. Combined allylamine and egg-yolk lesion. Twelve days. Lipophages now form a subendothelial xanthoma, with hemorrhage and newly formed blood channels. x300.



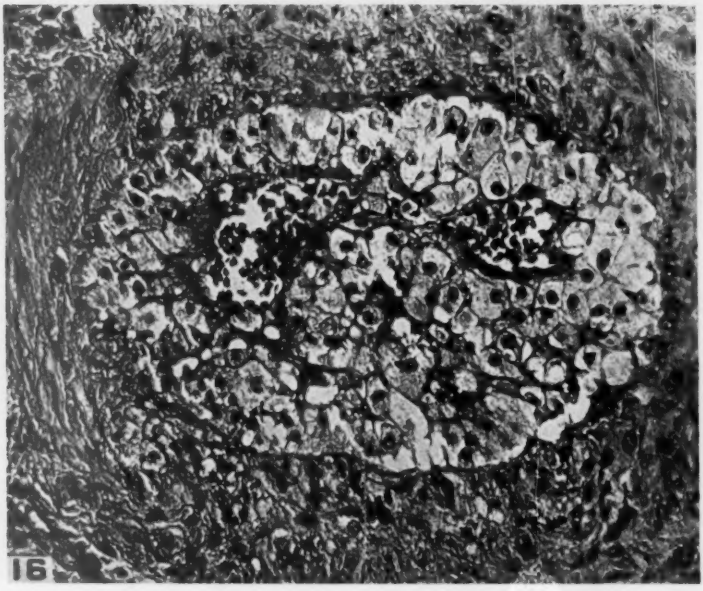
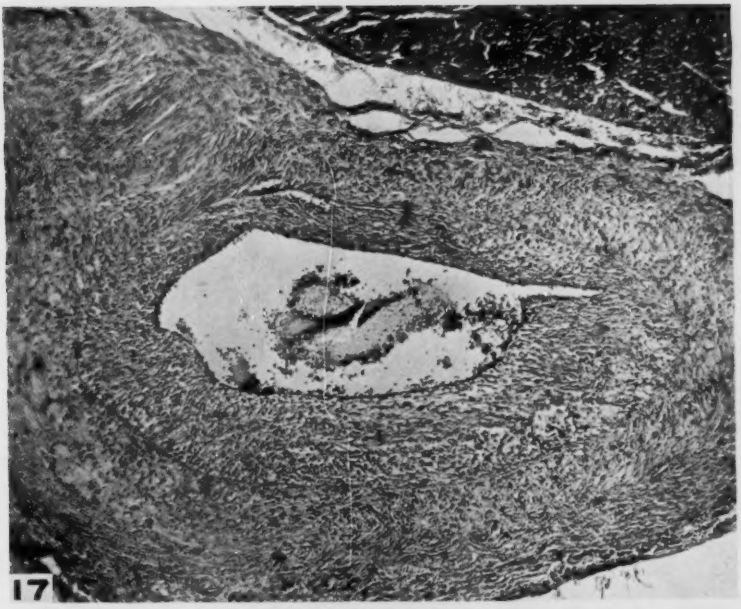


FIG. 15. Dog 1087. Coronary artery. Fully developed intimal fatty plaque, eleven days after commencement of allylamine-egg-yolk regimen. x220.

FIG. 16. Dog 1096. Coronary artery. Combined allylamine-egg-yolk regimen, eleven days. The intimal xanthoma is fully developed. Stigmata of previous inflammation are lacking. x350.



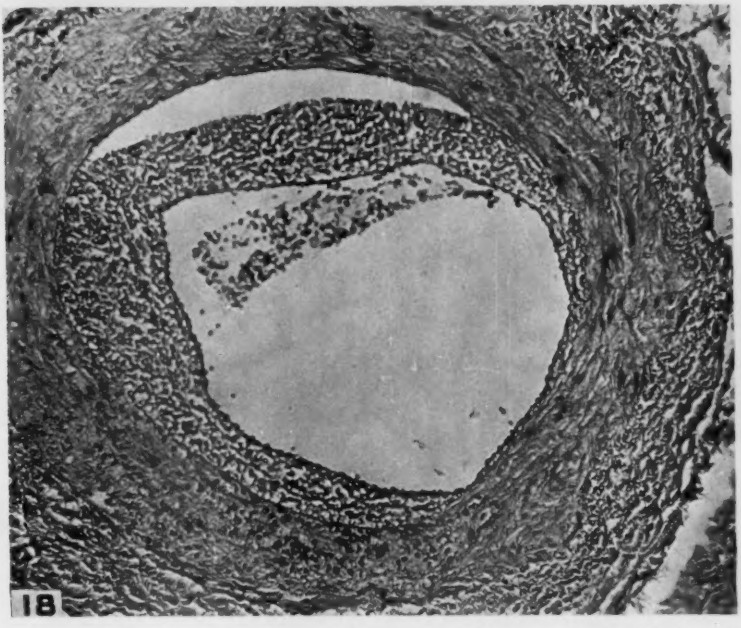
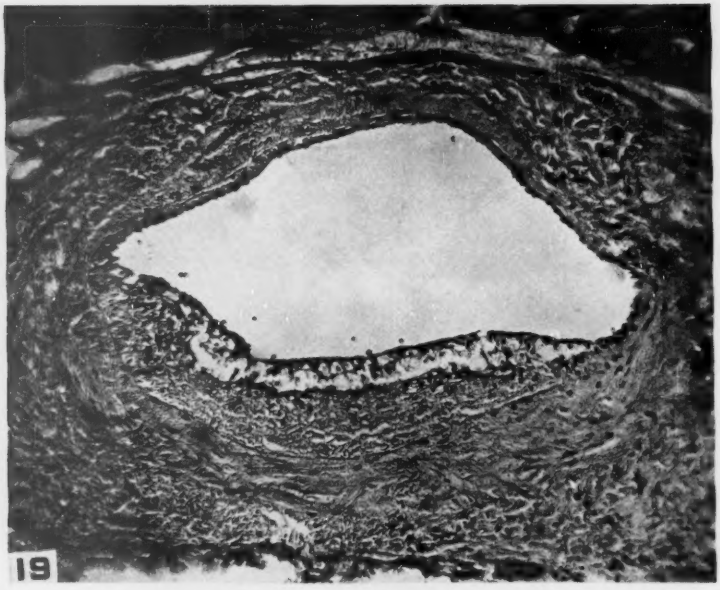


FIG. 17. Dog 1132. Coronary artery, three weeks after a course of allylamine and egg-yolk. The fatty intimal plaque is being invested by fibrous connective tissue. Foam cells remain in areas adjacent to the internal elastic membrane. x175.

FIG. 18. Dog 1132. Coronary artery. Completely "regressed" foam-cell lesion three weeks after a course of allylamine and egg-yolk. The lipophages have disappeared and the plaque has progressed to scar. x100.



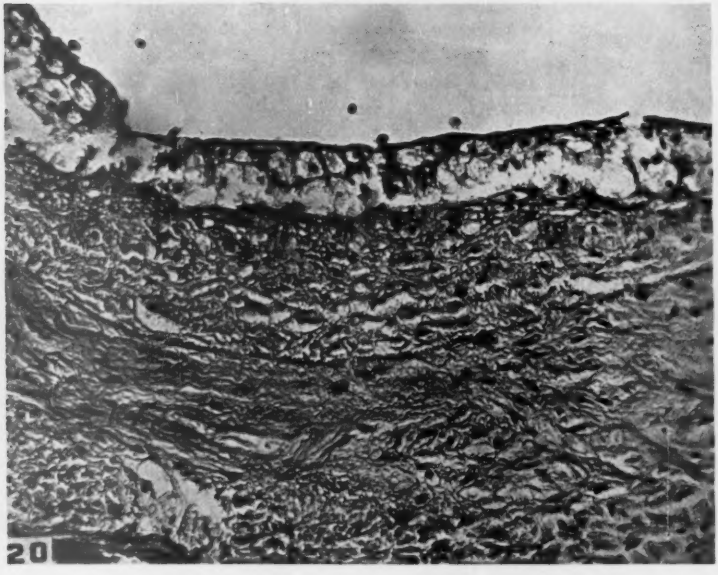


FIG. 19. Dog 1194. Egg-yolk alone. Fresh foam-cellular lesion superimposed on intimal scar of coronary artery previously damaged by allylamine. x140.

FIG. 20. Dog 1194. Higher magnification of Figure 19. Lesion confined entirely to intima. x325.

The impression was gained by comparing many control lesions of allylamine with the lesions of combined allylamine and lipid administration that the fatty intimal granulomas of the latter were larger and were more occlusive.

The effect of establishing the experimental conditions after progression of the control lesion to scar

Dogs were given allylamine in the usual dosage schedule. Two to three months later, at a time when the endarteritic, non-fatty lesions were converted to scar, a course of intravenous egg-yolk alone or of allylamine and egg-yolk was given. Histological examination of the coronary arteries of these animals after appropriate intervals revealed that new lesions had occurred in both groups particularly at the sites of original injury. The lesions were more frequent and more extensive in the animals receiving allylamine and egg-yolk than in those given egg-yolk only. Infiltration of lipid into the vessel wall at scarred sites was now chiefly intimal rather than medial. No inflammation or degeneration occurred *in the depths* of the previously formed intimal scar, that might simulate the location of atheroma in man. A new foam-cellular process was instituted *on the surface* of the old intimal lesion. The endothelium became elevated and beneath it a foam-cellular process developed, entirely analogous to that developing in previously unaltered arteries (Figs. 19, 20). This lesion in turn progressed to scar. Thus recurrent fatty granulomas may lead to successive layers of intimal connective tissue that progressively reduce the lumen of an artery. An antecedent thrombus is not necessarily a part of this process.

DISCUSSION

The technique described for the experimental production of lesions of the coronary arteries and aortas of dogs presents certain advantages for the study of local and hemic factors in the pathogenesis of arteriosclerosis. The animal of choice is not prone to spontaneous vascular disease. The procedure is technically simple. The arterial lesions that result are numerous in any one animal and are fully developed within a week to ten days from the beginning of the experiment. The inflammatory focus in the artery wall that follows the injection of allylamine is a basic acute reaction of arteries that follows a variety of noxious stimuli.[84] It is not in any sense an unusual or unique process, and its course may be followed. Egg-yolk, injected to provide gross alterations in the quantities and physical form of lipids in the blood, is well tolerated by dogs and does not lead to severe anaphylactic

phenomena. Further, it contains a large quantity of particulate lipid in very fine emulsion and also in solution a known phospholipoprotein, lipovitellin. In addition, egg-yolk contains approximately 1.5 per cent cholesterol, almost all in the free form. High levels of cholesterol in the blood including cholesterol ester result promptly with repeated injections.

The anatomical lesions in any one animal present a panel of quantitative variations that are useful in determining the relative importance of local phenomena. Also the type and amount of lipid in the blood can be varied. For example, besides the effect of egg-yolk on basic arterial lesions, the effect of alimentary chylomicrons of dog or human source and of human plasma lipoproteins have been investigated[23] and will be reported in detail subsequently.

The selective localization of lipids in the artery walls at areas of injury emphasizes the dependence of this phenomenon on the presence of local increase in permeability. Thus no lipid could be found in normal dogs' coronaries or in the uninjured segments of allylamine-treated animals' vessels in spite of enormously elevated blood lipid values. Lipid did localize in dogs' aortas after egg-yolk injections alone, without prior administration of allylamine. However, it is known that the vessels of such animals may be injured by the circulatory overload involved, and therefore they could not be considered normal.[25] The selective localization of lipids from the blood at sites of arterial inflammation would appear to be a process paralleling the localization of certain dyes in areas of increased endothelial permeability, as observed by Menkin.[14]

The use of an acute inflammatory reaction of the vessel wall as a model for the study of the pathogenesis of arteriosclerosis may be criticized on the grounds that it differs markedly in histological appearance from the usual early arteriosclerotic lesion in man. This morphological discrepancy is transitory. The stigmata of previous inflammation in the experimental lesion disappear within ten days to two weeks; it becomes a fibrous intimal plaque laden with lipid-filled cells. At this stage the experimental lesion and small arteriosclerotic lesions in man present many features in common. It would be unwise to conclude from such evidence that the arteriosclerotic lesion is the result of a mural inflammatory process in the usual morphological sense, although evidence of cellular exudate is less rare in such lesions than is commonly thought. However, the experimental inflammatory lesion makes available for study segments of artery wall with increased permeability to plasma and to its contained particulate lipids and soluble lipoproteins. It presents sequences of intimal proliferation and of phagocytosis of fatty substances derived from the blood, as well as the subsequent

progression of these lesions to scar. Each of these processes may play a part in the development and progression of the characteristic lesion in man.

The failure of the lesions to continue in an active stage after the cessation of the experimental regimen is perhaps disappointing if one thinks of arteriosclerosis in man as a continuous, progressive process. However, the progression of the experimental lesions to scar indicates only that the injury involved was episodic in nature. Thus, if the experimental regimen was reinstituted, a new lesion occurred, superimposed on the original site. The pleomorphism of the lesions in man, including foam-cell areas superimposed on areas of scar,[26] the disappearance of fat from the lesions and their conversion to scar in children[25] and in emaciated individuals[27] may actually be evidence of similar episodic development of the lesions in man.

How lipid from the blood can gain entrance to the artery wall in arteriosclerosis has been much discussed.[28] In normal or in allylamine-injected dogs intravenous injection of egg-yolk resulted in the appearance within minutes to hours of sudanophilic material beneath the endothelium of the aorta and within the connective tissue spaces of the inner media. When the animals were sacrificed serially, deeper and deeper penetration of the media by diffusable lipid was observed. It was thus suggested that infiltration from the intima had occurred. Further, in neither the normal nor in the allylamine-injected animals was medial hemorrhage or necrosis a component of the aortic medial lesion. In the coronary arteries, where medial necrosis and hemorrhage were prominent, there can be no doubt that lipid-rich plasma in part reached the interior of the vessel, including even the subendothelial space, by dissection from the ruptured capillaries of the vasa vasorum. Another observation of interest was the limited period during the inflammatory reaction in which a particular area would take up lipid. This indicates the importance of changes in permeability, however mediated, in initiating and limiting the extent of lipid deposit within the artery wall and suggests again the possible episodic genesis of the lesion.

Study of the xanthomatous stages of the experimental lesion yields information concerning the localization and fate of lipids in arteries. Lipids appeared in the vessel wall first as diffusely stained, sudanophilic, extracellular areas. Rapidly droplets or globules of more intensely staining material accumulated. Phagocytosis began within two to four days from the time of entry of the lipid and progressed with the organization of the lesion. Large mononuclear cells present in the exudate at first took up the lipid. Later it was found in new fibrocytes as well. While some mononuclear cells containing lipid appeared to wander out of the lesion and disappeared via the lymphatics, many others more probably were associated with the elab-

oration of collagen and became transformed into fixed-tissue foam cells. Once maturity of the xanthoma was reached, disappearance of the phagocytized lipid was progressive and rapid. The digestion of the deposited lipid was intracellular and was complete after several weeks. Such phagocytosis may also result in the removal of lipid from the lesions of arteriosclerosis. Regressing foam cells similar to those of the experimental lesion are found in arteriosclerosis in man. The rapidity with which the experimental lesions progressed from initial edema, through fatty granuloma to scar, was striking. Even though the period was short, the impression was gained that scar formation was delayed by the presence of lipid in the lesion. Also there was evidence that the fatty granulomas of the combined procedure were frequently larger than the proliferative intimal lesions that followed allylamine injection alone. It would thus seem that the presence of lipid in intimal lesions increases the danger of functional impairment of the vessel.

Thrombi and myocardial infarction associated with the lesions were rare. This is remarkable considering the extensive necrosis that occurred and the thinness and delicacy of the intima. However, as reported, the endothelium usually remained intact, though often elevated. The occasional deposit and re-endothelialization of fibrin strands to form subendothelial spaces containing plasma elements and foam cells is interesting in view of Duguid's[5] observations on the possible rôle of fibrin in the pathogenesis of arteriosclerosis.

Vascularization of the experimental lesions was from downgrowth of vasal vessels of the media. Capillaries remained patent in fibrotic intimal lesions for long periods.

When the effect of lipid injection alone or of allylamine and egg-yolk was tested on the intimal scars of previously damaged vessels, no atheromatous degeneration took place deep within the fibrous plaques. Superficial inflammation did occur, giving rise to superimposed fatty granulomas that developed and regressed in a fashion similar to granulomas occurring in previously unscarred vessels. These recurrent lesions were purely intimal and occurred without the accompaniment of any fresh medial inflammation or necrosis. Also it is of interest that these lesions occurred preferentially at the sites of previous damage, suggesting that once scarred, the arterial intima possesses a lowered threshold for injury.

SUMMARY

An experimental technique for the rapid production of fatty intimal lesions of the coronary arteries and aortas of dogs is presented. It involves the combined intravenous administration of allylamine and of saline emulsions of egg-yolk. The procedure allows control and variation of certain

local and hemic factors important for the development of the arterial changes. Study of conditions associated with the establishment of the experimental lesions and observation of their course has yielded information of interest for the pathology of arteriosclerosis.

REFERENCES

1. Anitschow, N.: Über die Atherosklerose der Aorta beim Kaninchen und über deren Entstehungsbedingungen. Beitr. path. Anat., 1914, *59*, 306.
2. Bloor, W. R.: Studies on blood fat. I. Variations in the fat content of the blood under approximately normal conditions. J. biol. Chem., 1914, *19*, 1.
3. De Suto-Nagy, G. I. and Waters, L. L.: The effect of altered lipid metabolism on experimental lesions of the coronary arteries. Circulation (N. Y.), 1951, *4*, 468 (abstract).
4. De Suto-Nagy, G. I. and Waters, L. L.: Interrelationship of experimental coronary lesions and altered lipid metabolism. Fed. Proc., 1952, *11*, 413.
5. Duguid, J. B.: Pathogenesis of atherosclerosis. Lancet, 1949, *257*, 925.
6. Hawk, B., Oser, B. L., and Summerson, W. H.: *Practical Physiological Chemistry*, 12th Edition. Philadelphia, The Blakiston Co., 1947, pp. 541 *et seq.*
7. Jukes, T. H. and Kay, H. D.: Egg-yolk proteins. J. Nutr., 1932, *5*, 81.
8. Kelly, F. B., Taylor, C. B., and Hass, G. M.: Experimental atheroarteriosclerosis. Localization of lipids in experimental arterial lesions of rabbits with hypercholesterolemia. Arch. Path., 1952, *53*, 419.
9. Kelly, F. B., Jr., Taylor, C. B., Hass, G. M., and Craig, J. M.: Arteriosclerotic lesions in areas of vascular injury in hypercholesterolemic rabbits. Circulation (N. Y.), 1951, *4*, 463 (abstract).
10. McAllister, W. B. and Waters, L. L.: Vascular lesions in the dog following thyroidectomy and viosterol poisoning. Amer. Heart J., 1949, *38*, 463 (abstract) and Yale J. Biol. Med., 1950, *22*, 651.
11. McAllister, W. B. and Waters, L. L.: Unpublished experiments, 1950.
12. McAllister, W. B. and Waters, L. L.: The localization of particulate fat in injured blood vessels. Trans. N. Y. Acad. Sci., 1951, Series II, *14*, 83.
13. Man, E. B. and Gildea, E. F.: A modification of the Stoddard and Drury titrimetric method for the determination of fatty acids in blood serum. J. biol. Chem., 1932, *99*, 43.
14. Menkin, V.: *Dynamics of inflammation*. New York, The MacMillan Co., 1940, 244 pp.
15. Ophüls, W.: The pathogenesis of arteriosclerosis. In Cowdry, E. V., *Arteriosclerosis*. New York, The MacMillan Co., 1933, pp. 249-270.
16. Roe, J. H. and Kahn, B. S.: The colorimetric determination of blood calcium. J. biol. Chem., 1929, *81*, 1.
17. Schlichter, J. G., Katz, L. N., and Meyer, J.: The occurrence of atheromatous lesions after cauterization of the aorta followed by cholesterol administration. Amer. J. med. Sci., 1949, *218*, 603.
18. Schmitt, H.: Experimentelle Untersuchungen zur Pathogenese der rheumatische Atherosklerose. Lipoidablagerung im Allergie- (Hyperergie-) Schaden der Gefässwand. Virchows Arch. path. Anat., 1936, *296*, 603.
19. Schoenheimer, R. and Sperry, W. M.: A micromethod for the determination of free and combined cholesterol. J. biol. Chem., 1934, *106*, 745.
20. Taylor, C. B., Cox, G. E., Hall-Taylor, B. J., and Nelson, L. G.: Atherosclerosis in areas of vascular injury in monkeys with mild hypercholesterolemia. Circulation (N. Y.), 1954, *10*, 613 (abstract).

21. Waters, L. L.: Changes in the coronary arteries of the dog following injections of allylamine. Amer. Heart J., 1948, *35*, 212.
22. Waters, L. L.: Localization of lipids in injured coronary arteries of dogs following injection of egg-yolk fractions or of hyperlipemic human plasma. Circulation (N. Y.), 1953, *8*, 437 (abstract).
23. Waters, L. L.: Plasma lipids and the arterial wall. Circulation (N. Y.), 1954, *10*, 614 (abstract).
24. Waters, L. L.: The reaction of the artery wall to injury by chemicals or infection. National Academy of Sciences-National Research Council, Publication 338, Symposium on Atherosclerosis, 1955, pp. 91-98.
25. Waters, L. L. and de Suto-Nagy, G. I.: Circulatory factors in the pathogenesis of experimental arteriolar necrosis. Yale J. Biol. Med., 1950, *22*, 753.
26. Waters, L. L. and Duff, R. S.: The rôle of arterial injury in the localization of methyl cellulose. Amer. J. Path., 1952, *28*, 527 (abstract).
27. Wilens, S. L.: The absorption of arterial atheromatous deposits in wasting diseases. Amer. J. Path., 1947, *23*, 793.
28. Winternitz, M. C., Thomas, R. M., and LeCompte, P. M.: *The biology of arteriosclerosis*. Springfield, Ill., Charles C Thomas, 1938. 142 pp.

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THE EFFECTS OF METABOLIC ALTERATIONS ON EXPERIMENTAL RHIZOPUS ORYZAE (MUCORMYCOSIS) INFECTION§

Mucormycosis is a fungus infection which generally occurs in association with metabolic disorders, most frequently diabetes mellitus. The fungus has only recently been identified in culture when the phycomycetes Rhizopus oryzae and Rhizopus arrhizus were recovered from two diabetic patients with cerebral mucormycosis.[2, 10] With these and other species of Mucorales, lesions closely resembling cerebral and pulmonary mucormycosis in man have been produced in rabbits with acute alloxan diabetes while non-diabetic animals showed only rare minute lesions at the site of inoculation.[5, 9] Clinical, postmortem and experimental findings indicate, therefore, that severe metabolic alterations are essential in the pathogenesis of this fungus infection.[1, 5]

To define the metabolic factors more accurately the following experiments were carried out. The effect of sustained hyperglycemia in rabbits without diabetes was studied since elevation of blood sugar is one of the principal metabolic abnormalities in diabetes. We had also noted previously that the fungus could be isolated in culture from non-diabetic rabbits without lesions as late as one week after inoculation. To test if experimentally induced metabolic alterations in a previously normal animal can change the fungus from a saprophyte to a pathogen, normal rabbits were inoculated with fungus at various times preceding the production of diabetes by alloxan administration.

FUNGUS INOCULATION PRECEDING ALLOXAN

METHODS

The details of the experimental procedures are the same as previously described.[5]

A standardized suspension of Rhizopus oryzae spores was instilled into one nostril of 19 male rabbits.¶ Acute diabetes was produced by alloxan injection which was given

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to five rabbits 24 hours and to nine rabbits 96 hours after fungus inoculation. Table 1 shows the blood sugar values at 48 hours after alloxan administration and on the day of death. Acetonuria is recorded qualitatively. Complete autopsies including fungus cultures of nose and lung were performed on all animals either when moribund or within two hours after death. Five inoculated rabbits received no alloxan and served as controls. They were sacrificed at intervals corresponding to the survival times of diabetic animals.

TABLE 1.

RHIZOPUS ORYZAE INOCULATION PRECEDING ALLOXAN BY ONE AND THREE DAYS

RABBIT NUMBER	BLOOD SUGAR	URINE ACETONE	SURVIVAL HOURS AFTER ALLOXAN	FUNGUS LESIONS			FUNGUS CULTURES	
				NOSE	LUNG	BRAIN	NOSE	LUNG
ONE DAY								
872	644	−	60	+	+	?+	+	+
873	532 868	+	124	+	−	+	+	+
889	924 924	+	102	+	+	?+	−	+
890	556 −	+	94	+	+	+	+	+
891	512 776	+	128	+	+	+	+	−
THREE DAYS								
827	366 552	+	120	+	+	−	+	+
828	398 560	+	120	−	+	−	−	+
830	360 426	+	96	−	+	−	+	+
831	318 −	Tr.	68	−	−	−	−	−
835	388 536	+	96	−	−	−	+	+
911	290 366	+	90	+	−	−	+	+
912	524 763	+	90	+	+	−	+	+
913	334 682	+	96	+	−	−	+	−
914	266 468	+	90	+	−	−	−	+

RESULTS

The data of this experiment are summarized in Table 1. All five rabbits which had been inoculated with fungus one day preceding alloxan administration showed nasal lesions. Pulmonary lesions were present in four of these animals. Definite meningoencephalitis occurred in three rabbits while slight meningeal inflammation without demonstrable mycelia was found in the two remaining animals. In the nine rabbits inoculated with fungus three days before alloxan injection, nasal lesions were found in five and pulmonary lesions in four. The latter occurred twice in association with nasal infection while in the other two animals the lesions were confined to the lung. In this group, no cerebral lesions were noted. In two rabbits, no lesions were found, although cultures from both nose and lung of one of

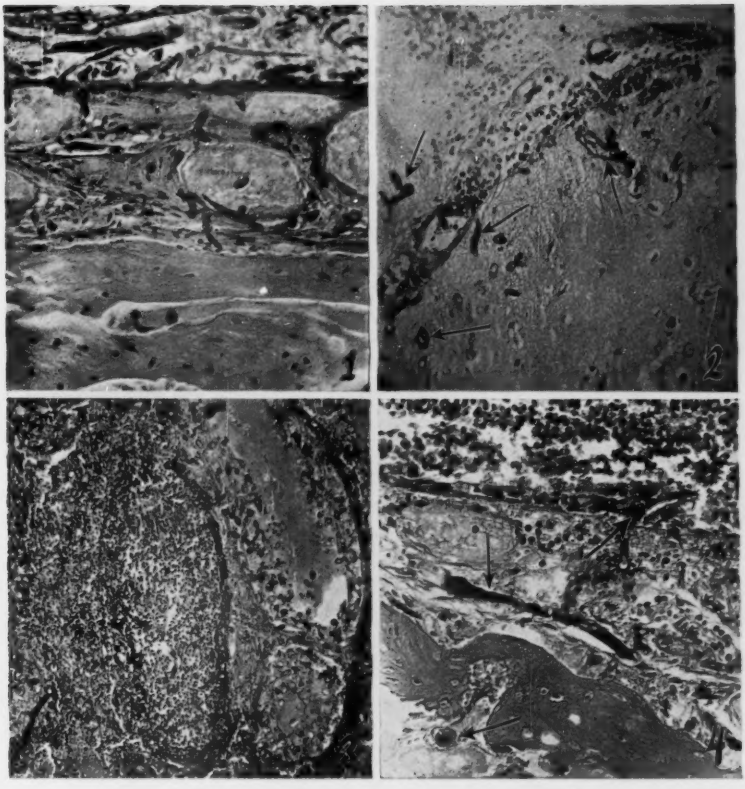


FIG. 1. Diabetic rabbit inoculated with fungus one day before alloxan administration. Extensive nasal lesion with mucosal ulceration, involvement of deep tissues, and many mycelia. Giemsa, x210.

FIG. 2. Same rabbit as Fig. 1. Early meningoencephalitis. Several mycelia are shown in the cortex (arrows). Giemsa, x180.

FIG. 3. Same rabbit as Fig. 1. Pulmonary lesion with involvement of bronchus and pulmonary artery. The vessel contains many mycelia. Giemsa, x115.

FIG. 4. Diabetic rabbit inoculated with fungus 3 days before alloxan administration. Extensive nasal lesion with bone necrosis. Note cross-section of mycelia in bone marrow (arrow) and other branching mycelia. Giemsa, x230.

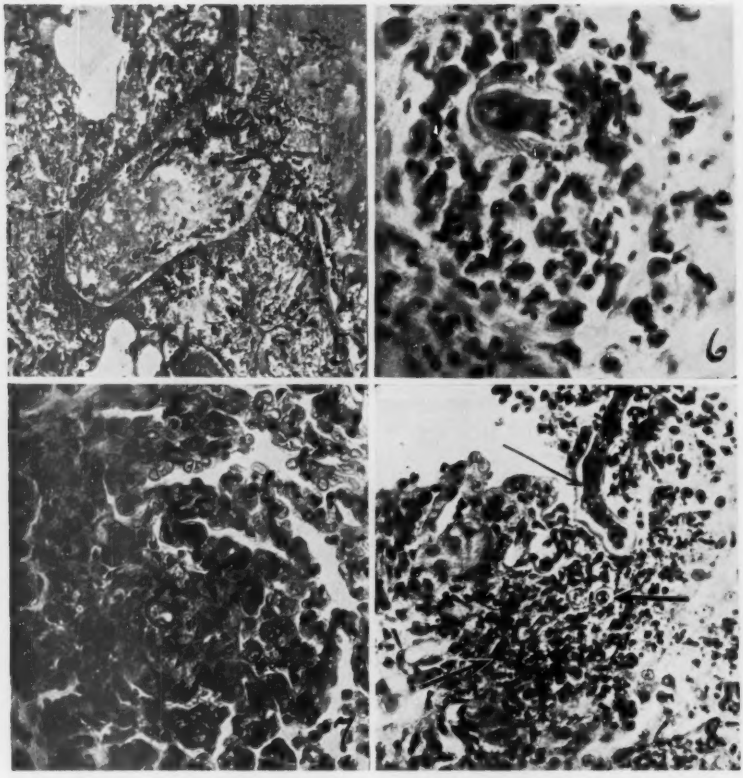
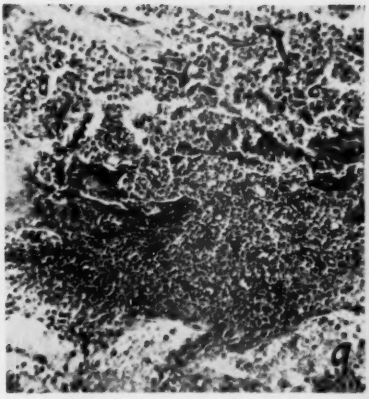


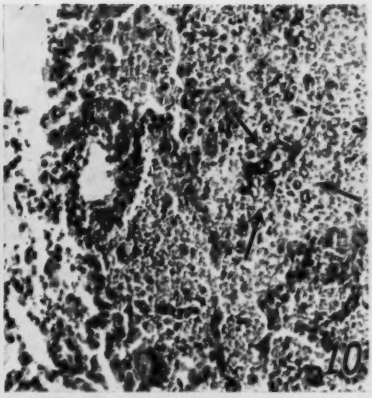
FIG. 5. Same rabbit as Figure 4. Extensive pulmonary lesion with many mycelia involving bronchus. Giemsa, x230.

FIG. 6. Rabbit with infusion hyperglycemia. Small nasal lesion (graded +) showing early hypha surrounded by inflammatory cells. Giemsa, x1000.

FIG. 7. Rabbit with infusion hyperglycemia. Pulmonary lesion (graded +) with degenerating spore surrounded by inflammatory cells. Giemsa, x440.

FIG. 8. Infusion hyperglycemia. Nasal lesion (graded ++) showing mucosal ulceration with several mycelia, some in cross-section (arrows). Giemsa, x400.





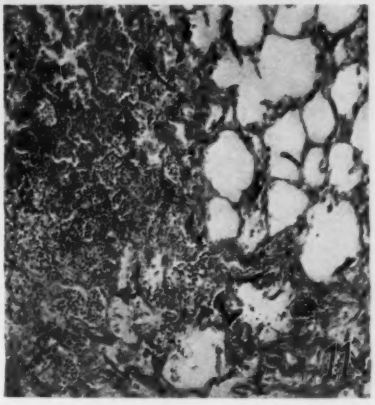


FIG. 9. Infusion hyperglycemia. Extensive nasal lesion (graded +++) involving deep structures and showing massive mycelial growth. Giemsa, x175.

FIG. 10. Bronchopneumonic focus of infusion hyperglycemia (graded ++) with cluster of mycelia (arrows). Giemsa, x220.

FIG. 11. Pulmonary lesion of infusion hyperglycemia with infarction of tissues and massive mycelial growth. Giemsa, x814.

these animals yielded fungus growth. Fungus cultures from the other rabbit without lesions showed no growth. Fungus cultures of nose and/or lung were positive in all other animals including the controls.

The fungus lesions in the diabetic animals resembled in every respect those reported previously.[8] The lesions were extensive and were characterized by marked mycelial proliferation and invasion of tissues, particularly blood vessels, which frequently produced infarction and led to dissemina-

TABLE 2.

INFUSION HYPERGLYCEMIA AND RHIZOPUS ORYZAE INOCULATION

RABBIT NUMBER	BLOOD SUGAR			SURVIVAL HOURS AFTER INOCULATION	FUNGUS LESIONS		FUNGUS CULTURES	
	MIN.	MAX.	MEAN		NOSE	LUNG	NOSE	LUNG
803	224	674	433	48	−	−	+	−
804	360	903	624	49	+	+	+	+
805	320	674	625	46	+	−	+	+
806	230	796	647	23	+ +	−	+	−
814	440	970	722	48	+++	+ +	+	+
815	290	636	461	49	+	−	+	+
816	340	670	565	49	+ +	+	+	−
817	414	690	536	49	+	−	+	+
866	378	520	466	45	+ +	−	+	−
867	422	616	510	48	+ +	−	+	+
868	436	623	467	49	+	−	+	+
869	218	404	342	50	+	−	+	−

tion. Degenerative changes in the polymorphonuclear leukocytes of the inflammatory reaction were another striking and constant finding. In the nose, there was infarction of tissue with bone necrosis and invasion of blood vessels and nerve trunks by numerous mycelia (Figs. 1, 4). The cerebral lesions in this series were quite early and consisted of invasion of leptomeninges and their vessels by fungus with beginning extension into the cerebral cortex which showed early infarction (Fig. 2). The pulmonary lesions consisted either of bronchopneumonic foci or of infarcted areas (Figs. 3, 5). In the latter, vessel invasion and occlusion by mycelial clusters dominated the picture.

The lesions in the non-diabetic controls were few and of minute size. They consisted of circumscribed foci of superficial ulceration of the nasal mucosa with many normal appearing polymorphonuclear leukocytes surrounding rare degenerating spores.

INFUSION HYPERGLYCEMIA

METHODS

The blood sugar determinations and inoculation procedures were the same as described previously.[8] The data of this experiment are summarized in Table 2.

Twelve male rabbits weighing between 2000-2500 gm. were given a constant intravenous infusion of 10% glucose in 0.45 saline with 20 mEq/L of potassium. The animals were immobilized on a stand with the neck secured in a yoke. They withstood the infusion well and showed no evidence of abnormal fluid retention except for mild dependent edema of the hindlegs. This usually appeared during the second day of the experiment. The infusion was given into the marginal ear vein through a 20-gauge hypodermic needle. The rate of infusion was adjusted on the basis of blood sugar determinations performed two to four times daily. Blood for biochemical determinations was obtained from the ear which was not the site of infusion. The minimum, maximum, and mean blood sugar levels are shown in Table 2. After blood sugar levels of more than 400 mgm% were reached, which occurred usually within two to four hours after the beginning of the infusion, the animals were lightly anesthetized with intravenous Nembutal and a standardized suspension of Rhizopus oryzae spores was instilled into one nostril. Hyperglycemia was maintained for 45-50 hours except in one animal which died 23 hours after inoculation because of technical difficulties. The other 11 rabbits were sacrificed by air injected into the infusion tubing after a final blood sample had been obtained. Complete autopsies including fungus cultures from the nose and lung were performed. The extent of the morphologic findings is shown graded from one to three plus in Table 2.

Plasma sodium, potassium, chloride, and CO_2 combining power determinations were performed daily during the experiment on rabbits Nos. 866-869 after base line values had been obtained for each animal. Plasma sodium and potassium were determined on the Baird flame photometer.* The method of Schales and Schales was used for the chloride determinations.[13] The microgasometric method of Scholander and Roughton was employed for estimation of the plasma CO_2 combining power.[14] All determinations were performed in duplicate. The values obtained in this manner checked closely and the mean values were recorded.

For controls, four rabbits were subjected to the same experimental procedures including electrolyte studies and determination of plasma CO_2 combining power but were not inoculated with fungus and received only 1.5 cc. of saline intranasally. These animals were sacrificed at the end of 48-50 hours when the infusion was terminated. Six other rabbits were given glucose infusion for 48-50 hours under the same experimental conditions but were not inoculated with fungus or saline. They were permitted to survive from 10-16 days after termination of the infusion at which time they were sacrificed and complete autopsies were performed. Blood sugar levels were determined before the infusion was discontinued, again 8-12 hours later and several times thereafter. Two additional rabbits were immobilized for 50 hours in the same manner as the other experimental animals but were neither infused nor inoculated. They were fed and watered without being removed from the stand. Plasma CO_2 combining power, Na, Cl, and K determinations were performed at 24 and 48 hours after base line values had been obtained. These animals were not sacrificed.

* We are indebted to Dr. Frank W. Fales, Department of Biochemistry, Emory University, for the plasma sodium and potassium determinations.

RESULTS

The data of this experiment are summarized in Table 2.

Nasal lesions were present in 11 of the 12 rabbits and pulmonary lesions were found in three of these. In six of the 11 animals, the nasal lesions (listed as one plus in Table 2) were few in number and consisted of minute superficial mucosal ulcerations. These showed viable-appearing, sometimes budding spores and hyphae (Fig. 6), and a vigorous inflammatory response by polymorphonuclear leukocytes which revealed marked nuclear pyknosis and sometimes karyorrhexis. In two of these six rabbits, rare minute foci of leukocytic exudate surrounding an occasional degenerating spore were found in the lung (Fig. 7), chiefly in alveolar ducts. The polymorphonuclear leukocytes of these lesions revealed the same nuclear changes as described above. In four other rabbits, the nasal lesions (graded as two plus in Table 2) were more numerous and extensive (Fig. 8). They consisted of circumscribed, but frequently confluent, areas of ulceration which extended beyond the mucosa into the deeper tissues. There was a vigorous response of polymorphonuclear leukocytes with purulent exudate in the nasal cavity. The leukocytes revealed pyknosis of nuclei and frequent karyorrhexis. Considerable mycelial proliferation was present in the purulent exudate of the nasal cavity and in the tissues with occasional mycelial invasion of capillaries, nerve trunks, and bone. There was no infarction of tissue and no pulmonary lesions were encountered. In another animal, widespread and marked nasal lesions (graded as three plus in Table 2) were seen to have formed by confluence of many small ulcerations. The deeper tissues, including bone and nerve trunks, were invaded by many mycelia (Fig. 9). Some capillaries and veins were invaded by fungus but neither involvement of arteries nor infarction of tissue was found. The inflammatory response was marked in the tissues and in the nasal cavity and consisted of polymorphonuclear leukocytes with large masses of mycelia. The nuclear alterations which have been described were present in the polymorphonuclear leukocytes of all lung lesions. On histological examination the purulent exudate of all nose lesions was found to contain some scattered bacteria. One animal showed no lesions. No cerebral involvement occurred in any of the rabbits. Cultures from the nose and/or lung yielded the fungus in all animals.

In one of the four control rabbits receiving intravenous glucose infusion and inoculated intranasally with saline, the nasal cavity showed on histological study a considerable amount of purulent exudate with large masses of bacteria, chiefly cocci and some bacilli. The mucosa revealed some small erosions confined to the uppermost layer with an infiltration by polymorphonuclear leukocytes. These cells showed the same regressive changes as seen

in the other hyperglycemic animals. The remaining three control animals showed no lesions.

All animals receiving glucose infusion revealed marked glycogen infiltration of the liver and of the renal tubular epithelium. The pancreatic islets appeared normal on routine histological study.

In the six control rabbits, with infusion hyperglycemia but without fungus inoculation, the blood sugar had returned to normal levels 8-12 hours after the infusion was discontinued and remained normal. The animals ate and acted normally, and autopsies performed 10-16 days after termination of the infusion revealed no lesions.

The electrolyte determinations in infected and control rabbits with hyperglycemia revealed a slight drop in plasma sodium within the first 24 hours. The values returned to base line levels at 48 hours. Plasma potassium determinations showed no significant changes. Plasma CO_2 combining power determinations at 24 hours showed a uniform fall of 20-25 vol.% from average base line values of 40-50 vol.%. The values at the termination of the experiment either remained the same or rose to 30-35 vol.%. After 24 and 48 hours, plasma chloride values were elevated about 10-15 mEq/L above average base line values of 95-105 mEq/L.

The two rabbits which served as controls of the effects of prolonged immobilization alone showed at 24 hours a drop in plasma CO_2 combining power of 8-12 vol.% with a rise towards normal values at 48 hours. Plasma Na, Cl, and K determinations revealed no significant changes.

DISCUSSION

Our findings re-emphasize the importance of metabolic alterations in the pathogenesis of Rhizopus oryzae infection.

Normal rabbits inoculated with fungus show only rare minute lesions at the site of inoculation, but fungus cultures from the tissues at autopsy are positive for as long as 13 days after inoculation. It appears, therefore, that the fungus persists in the tissues of the metabolically normal animal and that the host cannot readily destroy the agent. An aggressive fungus infection is, however, precipitated when acute alloxan diabetes is induced in rabbits which have been inoculated with fungus either one or three days before alloxan injection. In this experiment, all but one rabbit developed extensive lesions in one or several sites (Table 1).

The greater frequency and more extensive dissemination of fungus lesions among the rabbits inoculated one day prior to alloxan administration may result from the persistence of a larger number of viable spores as compared to the animals inoculated three days before alloxan injection. Rabbit No.

831 was injured while struggling during alloxan administration and died before diabetes with acidosis developed, which may account for the absence of lesions.

During this and previous experiments it had been noted by us and others that mucormycosis can be produced in rabbits with acute alloxan diabetes.[8, 9] Animals with chronic alloxan diabetes survive. They do not develop acidosis and are not susceptible to mucormycosis. Acute alloxan diabetes in rabbits is clinically quite similar to diabetes mellitus with acidosis and coma in man. This similarity includes the chemical changes which consist of glycosuria, hyperglycemia with ketosis, hyperlipemia, decreased plasma CO_2 combining power, hyponatremia, and dehydration.[8]

The second group of experiments shows that hyperglycemia produced by glucose infusion and not associated with diabetes induces changes in the host which render the fungus pathogenic. The lesions differ, however, in number, size, and appearance from those of the diabetic as well as the metabolically normal rabbits.

In six of 11 rabbits, the nasal lesions resembled those of the metabolically normal control animals in size and superficial location. They differed from the findings in the controls by an increase in frequency and the presence of viable, sometimes budding spores and hyphae. In two of the six animals, rare minute foci of bronchopneumonia with a few degenerating spores were seen, while with intranasal inoculation pulmonary lesions had previously been encountered only in diabetic rabbits. In another four of the 11 rabbits, the nasal lesions were still more numerous. They were also more extensive having formed by confluence of small mucosal ulcerations. The lesions showed mycelial proliferation and involvement of deeper tissues including nerve trunks and bone. In this respect, the findings resembled the lesions of diabetic rabbits from which they differed, however, by their more circumscribed appearance and by the absence of mycelial invasion of blood vessels with infarction of tissues. Occasional mycelia, however, were present in a few capillaries. In the 11th rabbit, the nasal lesions were still more widespread, approaching but not quite reproducing those of the diabetic animals. The ulcerations extended into the bone, and there was massive invasion by mycelia with involvement of nerve trunks, veins, and capillaries. The arteries, however, were spared, and there was no infarction. In the same animal, lesions were present in the lung which consisted of one area of infarction and many foci of bronchopneumonia. The infarcted area closely resembled similar pulmonary lesions in the diabetic animals. The bronchopneumonic foci differed from their counterpart in the diabetic rabbits in their smaller size, lesser number of mycelia, and the absence of vascular and bronchial wall invasion by fungus.

The inflammatory response in all lesions consisted chiefly of polymorphonuclear leukocytes which were numerous and uniformly showed marked nuclear changes of pyknosis and karyorrhexis. The degree of leukocytic infiltration and the nuclear alterations closely resembled those observed in the lesions of rabbits with acute alloxan diabetes. The lesions associated with infusion hyperglycemia may be regarded as representing an intermediate stage in the susceptibility of the host to Rhizopus oryzae infection. They are distinctly more frequent and active than the rare minute ulcerations of the metabolically normal animal but lack the aggressiveness, particularly the characteristic mycelial invasion of arteries with subsequent infarction of tissue which is seen in diabetic animals.

The electrolyte changes associated with infusion hyperglycemia consisted of hyperchloremia, decreased plasma CO_2 combining power and slight hyponatremia. These changes may be the result of an excess intake and excretion of chloride ions combined with a decrease in respiratory CO_2 excretion resulting from prolonged immobilization. In diabetes mellitus and probably also in acute alloxan diabetes, the acidosis largely results from a greatly increased utilization of fat and subsequent ketonemia which may be an important factor in the biochemistry of infection.[6]

The blood sugar levels produced by glucose infusion were generally the same as those of rabbits with acute alloxan diabetes and can, therefore, not account for the differences of the lesions in the two experiments. A significant effect of an increased glucose content of body fluids and tissues on the fungus is unlikely since similar fungus lesions can be produced in normoglycemic rabbits rendered agranulocytic with nitrogen mustard.[5] The metabolic changes leading to the formation of fungus lesions, therefore, appear to affect mainly the host. We have shown that despite certain similarities the lesions induced by infusion hyperglycemia differ from those produced in animals with acute alloxan diabetes. In both states of altered metabolism, the polymorphonuclear leukocytes in the lesions display similar degenerative changes which suggest impaired leukocytic function. Other investigators have shown that the function of the leukocyte is affected by metabolic disturbances.[12] Martin and associates have demonstrated *in vitro* that the polymorphonuclear leukocytes of diabetic patients have impaired glycolytic power and form less lactic acid.[11] This may reduce the bactericidal capacity of the leukocyte and can be reversed by the addition of insulin. The findings of Cruickshank and Payne indicate that the bactericidal power of the leukocyte in the alloxan diabetic rabbit is impaired.[7] In another study, Cruickshank attributes the increased susceptibility to infection of the diabetic animal to peripheral circulatory failure which inhibits the migration of leukocytes into the infected tissues.[8] In our experiments, we have found no

difference in the degree of leukocytic reaction in the fungus lesions associated with either infusion hyperglycemia or acute alloxan diabetes. Cruickshank's observations, however, refer to bacterial skin lesions which may account for some of the differences between his and our results.

Our findings suggest that the metabolic similarities and differences of infusion hyperglycemia and acute alloxan diabetes are related to the morphological resemblances and dissimilarities of the fungus lesions. Hyperglycemia and decreased plasma CO_2 combining power together with mycelial proliferation in the tissues and degenerative nuclear changes in the leukocytes are encountered in infusion hyperglycemia as well as in acute alloxan diabetes. Ketonuria, hyponatremia, hyperlipemia, dehydration, and massive fungus lesions with extensive blood vessel invasion and infarction of tissues occur only in acute alloxan diabetes. The changes in the polymorphonuclear leukocytes appear to be a common denominator in the lesions of both metabolic disturbances and suggest that altered leukocytic function is an important factor in the pathogenesis of Rhizopus oryzae infection. Distinct differences in the appearance of the lesions and in the behavior of the infection exist between infusion hyperglycemia and acute alloxan diabetes which, however, cannot be entirely explained by the changes in the leukocytes and indicate that other, as yet unrecognized, factors are present. These include the biochemical alterations of acute alloxan diabetes other than hyperglycemia and the mechanism of their effect on host resistance and will be the subject of further studies.

SUMMARY

Rabbits inoculated with Rhizopus oryzae will develop the lesions of nasal, pulmonary, and cerebral mucormycosis even when the fungus inoculation precedes acute alloxan diabetes by several days. Rabbits with infusion hyperglycemia inoculated with Rhizopus oryzae develop fungus lesions in the nose and lung. These lesions are more frequent and active than those of metabolically normal rabbits but lack the aggressiveness found in the lesions of rabbits with acute alloxan diabetes and suggest an intermediate stage of host susceptibility. In both acute alloxan diabetes and infusion hyperglycemia the polymorphonuclear leukocytes show degenerative changes which suggest that altered leukocytic function is an important factor in the pathogenesis of this infection.

ACKNOWLEDGMENTS

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REFERENCES

1. Baker, Roger: Pulmonary mucormycosis. Amer. J. Path., 1956, *32,* 287.
2. Bauer, Heinz, Ajello, Libero, Adams, Elizabeth, and Useda, Domingo H.: Cerebral mucormycosis: Pathogenesis of the disease; Description of the fungus, Rhizopus oryzae, isolated from a fatal case. Amer. J. Med., 1955, *18,* 822.
3. Bauer, Heinz, Flanagan, John F., Sheldon, Walter H.: Experimental cerebral mucormycosis in rabbits with alloxan diabetes. Yale J. Biol. Med., 1955, *28,* 29.
4. Bauer, Heinz, Flanagan, John F., and Sheldon, Walter H.: Unpublished data.
5. Bauer, Heinz, Wallace, George F., and Sheldon, Walter H.: Data to be published.
6. Cruickshank, A. H.: Resistance to infection in the alloxan-diabetic rabbit. J. Path. Bact., 1954, *67,* 323.
7. Cruickshank, A. H. and Payne, T. P. B.: Anti-pneumococcal powers of the blood in alloxan diabetes in rabbits. Bull. Johns Hopkins Hosp., 1949, *84,* 334.
8. Dubos, René J.: *Biochemical determinants of microbial diseases.* Cambridge, Mass., Harvard University Press, 1954.
9. Elder, David T. and Baker, Roger D.: Pulmonary mucormycosis in rabbits with alloxan diabetes. Arch. Path., 1956, *61,* 159.
10. Harris, Jerome S.: Mucormycosis: Report of a case. Pediatrics, 1955, *16,* 857.
11. Martin, S. P., McKinney, G. R., Green, R., and Becker, C.: Influence of glucose, fructose, and insulin on the metabolism of leukocytes of healthy and diabetic subjects. J. clin. Invest., 1953, *32,* 1171.
12. Nungester, W. G. and Ames, Ada May: The relationship between ascorbic acid and phagocytic activity. J. infect. Dis., 1948, *83,* 50.
13. Schales, Otto and Schales, Selma S.: A simple and accurate method for the determination of chloride in biological fluids. J. biol. Chem., 1941, *140,* 879.
14. Scholander, P. F. and Roughton, F. J. W.: Microgasometric estimation of the blood gases: IV. Carbon dioxide. J. biol. Chem., 1943, *148,* 573.

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ANTIVIRAL CHEMOTHERAPY†

As is well known, effective chemotherapy is available for a large number of infectious diseases caused by bacteria multiplying extracellularly. Effective chemotherapy is also available for a considerable number of infectious diseases caused by intracellular agents. As shown in Table 1, typhoid fever and tuberculosis provide examples of treatable diseases caused by non-obligate intracellular bacterial agents. Diseases caused by rickettsiae, which are obligate intracellular parasites (e.g., epidemic typhus and Rocky Mountain spotted fever), respond satisfactorily to treatment with the various tetracyclines or chloramphenicol. Results obtained earlier with p-aminobenzoic acid were less impressive. Finally, treatment of psittacosis and lymphogranuloma venereum with the various tetracyclines also gives satisfactory results. Until recently the agents responsible for these and certain other diseases were classified with viruses. Actually, the Chlamydozoaceae, as this group of agents is now designated, seem to be more closely related to rickettsiae than to the so-called true viruses.

The broad spectrum of effectiveness of the tetracyclines suggests that the therapeutic effects may be obtained through interference with a metabolic mechanism, probably biosynthetic, common to a wide variety of microbes. On the other hand, the parasite selective activity of p-aminobenzoic acid, penicillin, and sulfonamides emphasizes differences among the agents of epidemic typhus, psittacosis, and lymphogranuloma venereum. It is likely that these differences are also metabolic in nature. Available evidence indicates that the chemotherapeutic agents listed in Table 1 reduce the rate of multiplication of the various infectious agents. It is entirely possible, however, that in some instances the infecting agent is killed in the presence of the chemotherapeutic compound and not merely retarded. As the result of either mechanism, the number of infecting particles is kept relatively low. When the number is kept below a critical value for a given infection, symptoms and signs of disease may disappear or remain in abeyance.[33]

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In sharp contrast, the multiplication of the so-called true viruses, i.e., agents of the size of vaccinia or smaller, is not slowed down or stopped by any presently useful chemotherapeutic drug. Furthermore, the course of disease caused by true viruses is not favorably altered by the administration of any known chemotherapeutic agent, except when secondary bacterial infection is also present.

It may be concluded that the site of multiplication is not a prime factor in determining chemotherapeutic effectiveness and that chemotherapy of

TABLE 1. CHEMOTHERAPY OF INFECTIOUS DISEASES CAUSED BY INTRACELLULAR AGENTS

Infectious agents	*Diseases*	*Useful chemotherapeutic agents*
Bacteria	Typhoid fever	Tetracyclines, chloramphenicol
	Tuberculosis	Streptomycin, isoniazid
Rickettsiae	Epidemic typhus Rocky Mountain spotted fever	Tetracyclines, chloramphenicol, p-aminobenzoic acid
Chlamydozoaceae	Psittacosis	Tetracyclines, penicillin
	Lymphogranuloma venereum	Tetracyclines, sulfonamides
Viruses	Mumps	.....
	Infectious hepatitis	.....

diseases caused by numerous intracellular agents, excepting the viruses, has already been accomplished.

The fact that a certain chemical substance inhibits one intracellular agent but not another suggests that the modes of reproduction of the two agents are different (Table 2). Available evidence, although scant, supports this idea along three lines: (i) The size of the smaller viruses makes it unlikely that they contain numerous complex enzyme systems. This may not apply to the larger viruses. (ii) Metabolism of glutamate and pyruvate, independent of host cell metabolism, has been demonstrated in typhus rickettsiae.[4, 5] Thus it is likely that rickettsiae possess an energy-yielding mechanism of their own. However, enzyme systems of carbohydrate metabolism were not demonstrated in purified feline pneumonitis elementary bodies, although the conditions of the experiment were similar to those under which typhus rickettsiae were investigated.[20] Feline pneumonitis agent belongs with the Chlamydozoaceae. Clearly, more work needs to be done in this area before a definitive statement can be made on the presence or absence of an independent energy-yielding mechanism in Chlamydozoaceae. Attempts to

demonstrate energy-yielding enzyme systems in true viruses have failed. Apparently, viruses are dependent on host cells not only for building blocks but also for the energy required in biosynthetic processes. (iii) Rickettsiae multiply by binary fission. There is some evidence that at one stage in the complex cycle of reproduction of Chlamydozoaceae, binary fission takes place involving the so-called initial bodies which are about twice the size of the elementary bodies.[48] When microbes multiply by binary fission, biosynthesis of new materials, including nucleic acids and proteins, takes place

TABLE 2. CHARACTERISTICS OF INFECTIOUS AGENTS

Infectious agents	*Chemotherapy available*	*Size mμ*	*Intracellular parasitism*	*Presence of energy-yielding metabolism*	*Mode of multiplication*
Bacteria	+	>500	Not obligate	+	Binary fission
Rickettsiae	+	300 x 700	Obligate	+	Binary fission
Chlamydozoaceae	+	300-450	Obligate	?	? (Partly binary fission)
Viruses	0	15-250	Obligate	0	Eclipse → reproduction of hereditary material → stepwise development of new particles.

inside particles which then proceed to divide. Division is preceded or accompanied by distribution of particle constituents among daughter particles. In contrast, where evidence is available, it indicates that synthesis of constituents of true viruses takes place outside, or rather in the temporary absence of, a limiting membrane. As the synthesis of materials is completed, component parts are formed and new particles assembled.

Thus it is likely that the reproduction of viruses is more intimately dependent on host cell metabolism than is the multiplication of the larger and more complex intracellular agents such as rickettsiae.

Many attempts have been made to inhibit virus multiplication without causing damage to the host. Most such attempts have been unsuccessful, probably because virus multiplication is so intimately dependent on host cell metabolism. In what follows, the results of some of the more successful experimental studies on viral chemotherapy will be described. Most of the observations will concern agents capable of slowing or stopping virus multiplication. However, in a number of instances to be referred to, the mechanism of action of the agent is not clearly established. Direct inactivating

effects on the infectivity of virus particles may be operative in some cases. Furthermore, certain compounds may exert an effect on tissue responses which make up the composite picture of disease. In selecting agents for review, two considerations were the main guides: (i) Promise shown in animal experiments and (ii) Possibilities of improvement of chemotherapeutic properties through chemical synthesis of new derivatives. A much more complete review, covering chemotherapy of bacterial, plant, and animal viruses, has recently appeared.[18] Among the agents to be discussed,

TABLE 3. EXPERIMENTAL CHEMOPROPHYLAXIS OR THERAPY OF VIRAL DISEASES WITH NATURALLY OCCURRING SUBSTANCES

Virus	*Test Animal*	*Substance*	*Author*
Pneumonia Virus of Mice (PVM)	Mouse	Capsular polysaccharide of K. pneumoniae	Horsfall and McCarty (1947);[14] Ginsberg and Horsfall (1951)[8]
MM	Mouse	M5-8450 (from Penicillium stoloniferum)	Powell *et al.* (1952, 1953)[23, 24]
Semliki Forest	Mouse		
Polio, Type 2	Mouse		
Polio, Type 1	Monkey	"	Cochran *et al.* (1954)[6]
Col SK Encephalomyelitis	Mouse	Helenine (from Penicillium funiculosum)	Shope (1953)[26–28]
Semliki Forest	Mouse		
Polio, Type 1	Monkey	"	Cochran and Francis (1955)[7]

several represent so-called naturally occurring substances, whereas others are synthetic chemicals.

Each of the naturally occurring substances listed in Table 3 is capable under certain conditions of protecting a proportion of experimental animals from death due to viral infection. However, it should be emphasized that the conditions under which protection is demonstrable are such as to render unlikely the possibility that these naturally occurring substances might be useful in the treatment of viral disease in man.

PVM is a latent agent in mice, and so far as is known, does not cause pneumonia in man. The capsular polysaccharide is effective only when given by the same intranasal route as the virus.[12, 14] Intranasal instillation of *K. pneumoniae* polysaccharide in amounts of 100 μg per mouse results in the development of pulmonary lesions.[8] In direct tests of chemotherapeutic effectiveness the polysaccharide modified the course of PVM pneumonia in the mouse and converted a virus infection which is rapidly fatal in control animals into a mild illness from which treated animals recovered.[8] This

result was secured with a total dose of 20 μg of *K. pneumoniae* polysaccharide per mouse when treatment was given two or three days after infection. At this dosage level the polysaccharide does not cause macroscopic lung lesions. Before the substance was given, considerable virus reproduction had occurred and definite pneumonia was already present. After the administration of the polysaccharide, further multiplication of the virus was inhibited and soon ceased entirely. At the same time the progress of the pneumonia was retarded and the lesions finally resolved. The *K. pneumoniae* polysaccharide had no effect on the multiplication of influenza A or B virus in the mouse or in the chick embryo.[33]

Most of the reported experiments with M5-8450, which is a crude filtrate from culture of *Penicillium stoloniferum,* were on chemoprophylaxis rather than therapy.[23, 24] The viruses used were inoculated intraperitoneally or subcutaneously or given by other peripheral routes. The available data indicate that M5-8450 injected intraperitoneally 24 hours before inoculation of virus prevents the death of a proportion of mice subsequently infected with MM, Semliki Forest or poliovirus type 2. The effectiveness of M5-8450 is much lower when it is administered by routes other than the intraperitoneal. It is ineffective when given 4 to 6 hours after virus inoculation. Furthermore, survivor mice, previously infected with MM or Semliki Forest virus and treated, were found to be susceptible to new infection with homologous virus.

In chemoprophylactic experiments in monkeys M5-8450 was injected in repeated 25 ml. amounts intraperitoneally.[8] The Mahoney strain of poliovirus type 1 was given subcutaneously. In monkeys which received a total dose of 100 ml. or more, the incidence of paralysis in control monkeys was 56 per cent (14 out of 25) and that in the treated group was 17 per cent (4 out of 24). The day of onset of paralysis in control monkeys ranged from 7 to 32, whereas in the treated group it ranged from 24 to 42. Thus treatment prolonged the incubation period. The authors concluded that in its present state of development M5-8450 did not appear to have direct therapeutic application.

Helenine is an acetone precipitate of an extract obtained from the culture pellicle of *Penicillium funiculosum.*[25–28] Mice that had been saved from either Col SK or Semliki Forest virus infection by treatment with helenine frequently were fully susceptible to reinfection by homologous virus. This was especially true if the infecting dose of virus from which they had been saved was a small one. With both viruses most significant effects were obtained in treating infections initiated by 10 to 100 fatal doses of either virus. Helenine had little if any effect on the course of infection when more than 1,000 fatal doses were inoculated. Careful studies of the relationship

between the amount of helenine injected and degree of protection obtained showed that dependence of the protective effect on dosage was demonstrable when relatively small amounts of helenine were used. A plateauing of effectiveness was observed at larger doses, i.e., beyond a certain point, increasing the dose did not increase the effect. For optimal effect helenine had to be given within 3 hours of the time of infection.

TABLE 4. EXPERIMENTAL CHEMOPROPHYLAXIS OR THERAPY OF VIRAL DISEASES WITH SYNTHETIC COMPOUNDS IN THE MOUSE

Virus	*Substance*	*Author*
Vaccinia	Phenoxypyrimidines	Thompson *et al.* (1951);[42] Bauer (1955)[3]
"	Benzaldehyde thiosemicarbazones	Hamre *et al.* (1950, 1951);[9,10] Thompson *et al.* (1951, 1953)[41,40]
"	Isatin thiosemicarbazones	Thompson *et al.* (1953);[39] Bauer (1955)[3]
"	Aliphatic oxime thiosemicarbazones	Thompson *et al.* (1953)[39]
Russian Spring-Summer Encephalitis	2,6-Diaminopurine	Moore and Friend (1951)[19]
Col SK Encephalomyelitis	2-Hydroxy-1,4-naphthoquinonimine	Schnitzler *et al.* (1951);[35] Jungeblut (1951)[18]
Influenza A, brain	α-Aminosulfonic acids	Ackermann (1952)[1]
Eastern Equine Encephalomyelitis	Quinacrine (atebrin)	Hurst *et al.* (1952)[15]
Influenza B, lung	5.6-Dichloro-1-β-D-ribofuranosyl-benzimidazole	Tamm *et al.* (1954)[37]

Helenine, like M5-8450, exerts a preventive effect on paralytic poliomyelitis due to the Mahoney strain of type 1 poliovirus in monkeys.[7] The incidence of paralysis was reduced from 100 per cent in the control group to 18 per cent in the treated group. Although this material had marked chemoprophylactic activity, no significant chemotherapeutic effect was seen in monkeys when treatment was withheld until the onset of the disease.

As Shope has pointed out, helenine may be related very closely to the active principle in M5-8450 and may even be the same substance.[36] The chemical nature of either material is not known. It has been shown that helenine is non-dialyzable.[36]

We turn now to results which have been obtained with synthetic compounds. Table 4 lists studies with a variety of viruses and compounds in the

mouse. In all of the studies listed with neurotropic viruses, protection from death of a proportion of infected animals was clearly demonstrated. To assess the importance of this remarkable finding it is necessary to take note of the experimental conditions under which protection was demonstrable.

It is of considerable interest that, in studies with vaccinia virus, administration of compounds in diet or subcutaneously protected mice against virus injected intracerebrally.[2, 39–43] Surviving animals which had been treated with isatin thiosemicarbazone were immune to reinfection.[2] Few data have been reported on the toxicity of the compounds used in studies with the vaccinia virus. Available data indicate that it was necessary to give nearly toxic amounts of compounds in order to demonstrate protection. Likewise, very little is known about the relationships between time of administration of the compounds and the effect obtained. In none of these studies was a chemotherapeutic effect demonstrated. Administration of the compound was usually started before inoculation of virus. Where the chemical was given after manifestations of disease had appeared, it failed to influence the infection in favor of the host. Another discouraging finding was that neither benzaldehyde thiosemicarbazone nor isatin thiosemicarbazone was effective against vaccinia virus infection in the rabbit.[39]

It is of interest that 2,6-diaminopurine[19] and atebrin[15] were effective when given 24 hours after the virus. In experiments with intraperitoneally injected 2,6-diaminopurine, protection against Russian Spring-Summer encephalitis was demonstrable when the virus was injected intraperitoneally but not when the intracerebral route was used. Similar findings in regard to route of administration and toxicity were made with 2-hydroxy-1,4-naphthoquinonimine in studies on Col SK encephalomyelitis in mice.[16, 25] Moreover, 2-hydroxy-1,4-naphthoquinonimine was effective only when given within one hour of virus inoculation. After treatment with this compound surviving animals were susceptible to reinfection. α-Aminosulfonic acids were effective in preventing encephalitis due to the neurotropic WS strain of influenza A virus only when introduced intracerebrally.[1] The amount of virus inoculated in these experiments apparently was small, since 13 to 20 per cent of untreated control mice also survived. The route of infection was intracerebral. Data on toxicity of α-aminosulfonic acids in mice were not given. Atebrin fed to mice inoculated intramuscularly or intracerebrally with the virus of Eastern equine encephalomyelitis protected a significant proportion of infected mice from death.[15] The most effective doses were near the toxic limit, however, and no effect was seen when the drug was administered after infection of the nervous system had become established. It is of considerable interest that in embryonated eggs atebrin had no effect on infection with this virus.

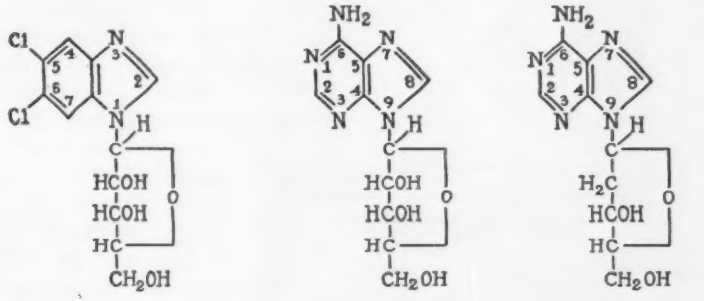
In our studies with influenza B virus in the mouse lung, we found that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, given in repeated intraperitoneal injections after intranasal inoculation of virus, retarded the multiplication of virus.[27] We found also (unpublished experiments) that this compound prolonged survival time but did not protect mice from death from pneumonia caused by the human influenza virus. Effective dose was close to the toxic dose. As is well known, a vast amount of work has been done with the influenza virus with the view of discovering effective inhibitors of this virus. Certain compounds have been found to inhibit multiplication of influenza virus in the chicken embryo without killing the embryo. However, most of these compounds have shown no effect on multiplication of this virus in the mouse lung. Thus, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole is among the few compounds which are capable of inhibiting influenza virus multiplication in the mouse lung and prolonging the survival time of infected mice.

The questions may be asked: (i) Why has it been relatively easy to demonstrate protection against neurotropic viruses in the mouse? (ii) Why has it thus far been impossible to protect mice against influenza virus pneumonia? No attempt will be made to answer these questions at this time, but it is suggested that the particular organ or organ system involved in a virus infection may condition the outcome of the experiment quite apart from susceptibility of the virus to inhibition by a chemical compound. It would seem desirable that more work be done on viral diseases involving organs other than the brain and lung. Now that viral hepatitis in the mouse is a fairly well-known disease,[23] it would seem indicated that intensive chemotherapeutic studies be undertaken on this model since the viruses of human hepatitis have failed to multiply and to cause disease in experimental animals.

To sum up the results thus far obtained and to assess their bearing on treatment of human or veterinary viral disease in the field, reference is made to Hurst,[15] who has stated that the requirements for a drug which would be useful in the field against arthropod-borne encephalitides are highly exacting and that experiments with atebrin do not suggest that it will meet these requirements. It would appear that this statement is applicable to all of the compounds and infections which have been discussed.

Clearly, if effective chemoprophylaxis or therapy is to be achieved, a great deal more work needs to be done in the laboratory. Continued screening of mold and other filtrates and of synthetic chemical compounds may yield effective agents, although returns from such efforts have not been abundant so far. The so-called rational approach has provided equally meager returns, probably because it has not been rational enough. We know essentially

nothing about intermediates in the biosynthetic pathways of animal virus nucleic acids or proteins beyond what we can guess. Yet it would seem that knowledge of the intermediates and of enzymes involved in biosynthesis is of the greatest importance for a rational approach to selective inhibition of virus multiplication. That interference with single specific chemical reactions involved in intracellular energy production does not provide a basis for selective inhibition of virus multiplication has been amply demonstrated. Such interference results in widespread disturbances in cellular metabolism and damage to host cells.



5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (Inhibitor)

9-β-D-Ribofuranosyladenine (In RNA)

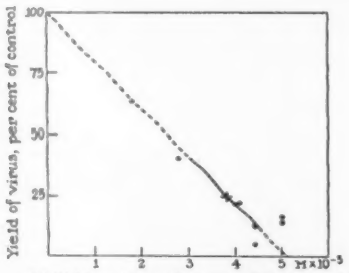
9-β-D-2-Deoxyribofuranosyladenine (In DNA)

FIG. 1. Comparison of the structure of an inhibitor of virus multiplication with the structures of two nucleosides.

The point of view which has been taken in our own work is that both qualitative and quantitative differences may exist between the biosynthesis of virus nucleic acids and host cell nucleic acids and that differences of either kind may provide a basis for selective inhibition of virus multiplication with appropriate compounds. However, approaches to chemical reactions which may be qualitatively specific for the multiplying virus, e.g., the hypothetical interlinking of nucleotides in a specific sequence to form virus nucleic acid, are not obvious at the present time. It appears that it may be more profitable to attempt exploitation of probable quantitative differences in the biosynthetic demands of the virus versus the host cell. We assume that the relative rates of synthesis of viral and host cell nucleic acids may be different and that differences may exist in respect to accessibility of sites of synthesis to inhibitor molecules. It is obvious that through the use of suitable chemi-

cal inhibitors, considerable insight might be gained into such differences.

Four years ago, in collaboration with Dr. Karl Folkers, we decided that benzimidazole derivatives might prove to be suitable as selective inhibitors of nucleic acid synthesis.[88] We proceeded to build up a body of knowledge of structure-activity relationships with already available and new compounds, and on the basis of such knowledge we made predictions as to what the structure of more effective compounds might be. Starting with simple alkyl derivatives of benzimidazole[88, 89] which showed low activity and selectivity, we have progressed to certain highly active and moderately selective ribosides of halogenated benzimidazoles.[89–93, 94, 97] The synthetic work has been carried out by Dr. Karl Folkers and Dr. Clifford H. Shunk of the Research Laboratories at Merck and Co., Inc.



	Molar concentration giving 75 per cent inhibition	Activity
Benzimidazole	0.0035	(1.0)
5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole	0.000036	(92)

FIG. 2. Determination of the inhibitory activity of benzimidazole derivatives on influenza B virus multiplication in the chorioallantoic membrane *in vitro*. Data from Tamm *et al.*[96, 97]

Figure 1 depicts the structural similarity between one of the highly active inhibitors, the β-D-ribofuranoside of 5,6-dichlorobenzimidazole, and adenine riboside. The similarity between the inhibitory riboside and adenine deoxyriboside is obviously less close. All of these compounds possess a bicyclic skeleton, and in each, one of the ring structures is imidazole. The pentose moiety is identical in the first two but different in the third.

In our work it was important to determine the inhibitory activity and selectivity of action, i.e., the activity-toxicity ratio, of derivatives with great precision, unhampered by problems of absorption, transport, and excretion. Also, since in most instances new compounds were used, it was important to be able to make such determinations on small amounts of material.

A technique was devised for the quantitative study of influenza B virus multiplication in the chorioallantoic membrane from embryonated chicken eggs *in vitro*.[95, 96, 97] Influenza virus was chosen mainly for reasons of experimental convenience. Yield of virus in the presence of compounds at inhibitory concentration was expressed in terms of per cent of yield in control

cultures. It was plotted against concentration of compound used (Fig. 2). The molar concentration required to cause 75 per cent inhibition of Lee virus multiplication was determined for each compound. For unsubstituted benzimidazole, this concentration is 0.0035 M. For purposes of comparison, benzimidazole has been used as the reference compound with an arbitrarily assigned Lee virus inhibitory activity of 1.

Figure 3 summarizes some important relationships between chemical structure and virus inhibitory activity.[20, 34, 37] As can be seen, the inhibitory activity of benzimidazole derivatives increased with multiple substitution of

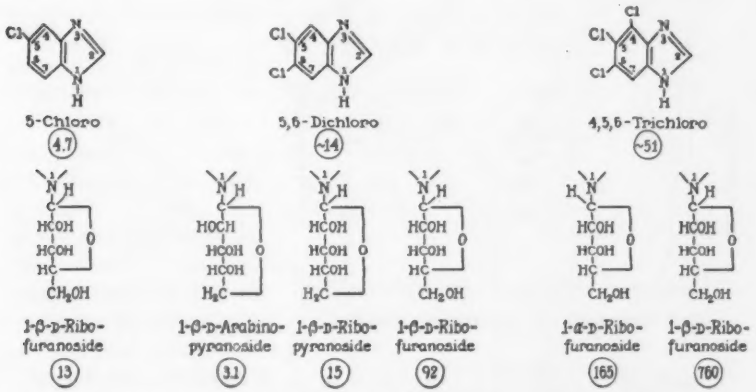


FIG. 3. Chemical structure and influenza B virus inhibitory activity of benzimidazole derivatives. Unsubstituted benzimidazole causes 75 per cent inhibition at 0.0035 M; activity = 1. The encircled numbers below each compound refer to relative activity. Data from Tamm,[20] and Tamm *et al.*[34, 37]

chlorine atoms in the benzenoid ring. Furthermore, the β-D-ribofuranosides of these chlorobenzimidazoles were much more active than the corresponding simple chloro derivatives (3, 7, and 15 times, respectively). When the glycosidic ring was changed from the 5-membered ribofuranoside to the 6-membered ribopyranoside, the inhibitory activity dropped to 1/6 of that found for the ribofuranoside; in fact, the activity of the ribopyranoside was almost identical with the activity of the simple 5,6-dichloro compound. The activity of the arabinopyranoside was even lower, indicating that substitution of this pentose caused inactivation of the chloro compound. Comparison of the α and β forms of the ribofuranoside of trichlorobenzimidazole showed that the β-linked ribofuranoside is much more active than the α form.

It has been reported that the nucleic acid in influenza virus is of the ribose type.[2, 17] Thus, the highest virus inhibitory activity is obtained when

the carbohydrate moiety at N1 is identical with that present in the nucleic acid of influenza virus particles and when the benzenoid ring is loaded with unnatural substituents.

Toxicity of compounds was studied by determining the concentration required to cause macroscopic changes in the chorioallantoic membrane on

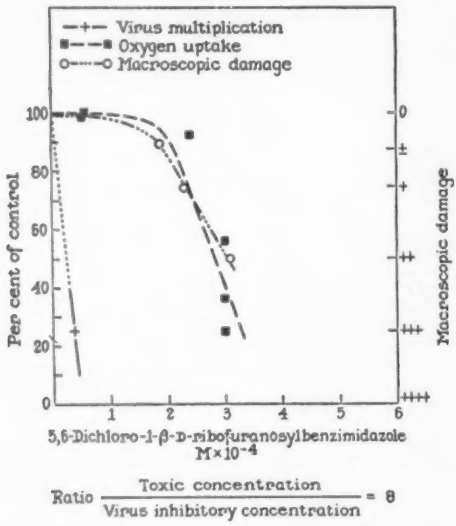


FIG. 4. Determination of influenza B virus inhibitory activity and tissue toxicity of benzimidazole derivatives in the chorioallantoic membrane *in vitro*. Toxic concentration = concentration causing 2+ macroscopic damage. Virus inhibitory concentration = concentration causing 75 per cent inhibition of virus multiplication. Data from Tamm.[20, 21]

Toxicity of compounds was studied by determining the concentration required to cause macroscopic changes in the chorioallantoic membrane on incubation *in vitro*.[20, 21] In the presence of toxic concentrations of compounds, the membranes became flaccid and unfolded, their color changed from pink to yellowish-white, and they lost weight. Figure 4 shows the relationship between macroscopic damage, reduced oxygen uptake, and inhibitory activity. The ratio of the concentration causing macroscopic alterations in the membrane to the concentration at which inhibition of virus multiplication occurs provides an indication of selectivity of action.

With key compounds, macroscopic and microscopic observations on membranes were supplemented with studies on the effect of compounds on oxygen uptake by host tissue, on proliferation of host cells in roller tube cultures, and on toxicity in animals. Results of such studies were in line with observations made on the chorioallantoic membrane *in vitro*.

In Table 5 the relationships between chemical structure and selectivity of action[20, 21, 24] are summarized. The 5-chloro compound, although 5 times more active than unsubstituted benzimidazole (as was shown in Fig. 3), was no more selective than the reference compound, as indicated by the identical ratios of toxic to virus inhibitory concentration. Clearly, substitu-

tion of the chlorine atom in the benzenoid ring caused a parallel increase in virus inhibitory activity and toxicity. In contrast, substitution of β-D-ribofuranose at N1 in the imidazole ring markedly increased virus inhibitory activity without increasing toxicity to the same extent. Thus the β-D-ribofuranose moiety confers selectivity of action on the molecule. The increase in selectivity is moderate. As can also be seen, β-D-arabinopyranose and β-D-ribopyranose failed to increase selectivity. Finally, to have this highly desirable effect on the molecule, ribofuranose must be present in the β-linkage.

TABLE 5. CHEMICAL STRUCTURE AND SELECTIVITY OF ACTION OF BENZIMIDAZOLE DERIVATIVES AS INHIBITORS OF INFLUENZA B VIRUS MULTIPLICATION*

Derivative	*Ratio* $\frac{\text{toxic concentration}}{\text{virus inhibitory concentration}}$
Benzimidazole	3
5-Chloro	3
5-Chloro-1-β-D-ribofuranosyl	8
5,6-Dichloro-1-β-D-arabinopyranosyl	3
5,6-Dichloro-1-β-D-ribopyranosyl	3
5,6-Dichloro-1-β-D-ribofuranosyl	8
4,5,6-Trichloro-1-α-D-ribofuranosyl	4
4,5,6-Trichloro-1-β-D-ribofuranosyl	20

* Data from Tamm,[20, 21] and Tamm *et al.*[24]

These findings show that with certain compounds virus inhibitory activity and toxicity vary independently. Through appropriate modification of the structure (i.e., substitution of β-D-ribofuranose), influenza virus inhibitory activity can be increased more than tissue toxicity. This selective effect is not obtained with chlorine atoms. However, it should be possible to replace chlorine atoms with some other substituents which, like β-D-ribofuranose, would confer selectivity on the molecule.

The special significance of the pentose moiety is emphasized by the finding[25] that β-D-ribofuranose, which is so important for selective inhibition of the ribonucleic acid-containing influenza virus, is not appropriate for inhibition of the deoxyribonucleic acid-containing[11, 22] vaccinia virus. We have found that substitution of β-D-ribofuranose in the chlorinated benzimidazole molecule causes no increase in inhibitory activity for vaccinia

virus. In Table 6 this finding is contrasted with observations made earlier with influenza virus.

It appears possible that for high activity against vaccinia virus and perhaps other DNA containing viruses, deoxyribosides of suitable benzimidazoles may be required.

It should be apparent that selective inhibition of influenza virus multiplication requires compounds of a special kind. Many of the benzimidazole derivatives and a series of vitamin analogs, as well as benzene, and purine derivatives which we have examined lack the properties which would recommend them for more extensive virus studies. The very high influenza virus inhibitory activity and the moderate selectivity of the β-D-ribofurano-

TABLE 6. EFFECT OF THE RIBOSE MOIETY ON THE INHIBITORY ACTIVITY OF CHLOROBENZIMIDAZOLES ON INFLUENZA B AND VACCINIA VIRUSES*

	Ratio of inhibitory activities of benzimidazole derivatives	
Virus	*5-(or 6-)Chloro-1-β-D-ribofuranosyl-5-Chloro-*	*5,6-Dichloro-1-β-D-ribofuranosyl-5,6-Dichloro-*
Influenza B	2.7	6.7
Vaccinia	0.96	0.69

* Data from Tamm and Overman.[80]

sides stand out and suggest lines of investigation to develop still more selective derivatives.

The available evidence suggests that β-D-ribofuranosides of chlorobenzimidazoles interfere with metabolic processes involving ribofuranosides. It is likely that synthesis of influenza virus RNA is inhibited on the nucleoside level. That synthesis of host cell RNA may also be affected to some extent is probable. The inhibitable processes take place early during the so-called latent period of the cycle of influenza virus multiplication.[80]

Finally, it should be emphasized that even if highly selective and potent inhibitors of the multiplication of various viruses are found or developed, their usefulness in the field may depend much on the characteristics of the diseases in question. Factors such as length of the incubation period, site or sites of infection, duration of illness, rôle of the immune response and other mechanisms of the host in recovery, stability of the infectious agents, and other factors will probably be of considerable importance in determining the outcome of therapeutic intervention. Perhaps the outcome of such intervention will depend more on how soon after infection it is feasible to start

treatment than on any other factor. In certain viral infections the interval between first symptoms and the time when maximal virus levels are reached is short. In such situations prevention only may be feasible. A long incubation period, i.e., a long interval between exposure resulting in infection and appearance of first symptoms, would greatly increase the chance of successful prevention. Whether chemoprophylaxis were worthwhile would obviously depend on the severity of the disease, the attack rate, considerations having to do with the effect prophylaxis might have on the development of active immunity, and other factors.

REFERENCES

1. Ackermann, W. W.: α-Aminosulfonic acids and viral propagation. Proc. Soc. exp. Biol., N. Y., 1952, *80*, 362.
2. Ada, G. L. and Perry, B. T.: The nucleic acid content of influenza virus. Austral. J. exp. Biol. Med. Sci., 1954, *32*, 453.
3. Bauer, D. J.: The antiviral and synergic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice. Brit. J. exp. Path., 1955, *36*, 105.
4. Bovarnick, M. R. and Snyder, J. C.: Respiration of typhus rickettsiae. J. exp. Med., 1949, *89*, 561.
5. Bovarnick, M. R. and Miller, J. C.: Oxidation and transamination of glutamate by typhus rickettsiae. J. Biol. Chem., 1950, *184*, 661.
6. Cochran, K. W., Brown, G. C., and Francis, T., Jr.: Antiviral action of a mold filtrate on experimental poliomyelitis in cynomolgus monkeys. Proc. Soc. exp. Biol., N. Y., 1954, *85*, 104.
7. Cochran, K. W. and Francis, T., Jr.: Effect of helenine on experimental poliomyelitis in mice and monkeys. J. Pharmacol., 1956, *116*, 13.
8. Ginsberg, H. S. and Horsfall, F. L., Jr.: Therapy of infection with pneumonia virus of mice (PVM); effect of a polysaccharide on the multiplication cycles of the virus and on the course of the viral penumonia. J. exp. Med., 1951, *93*, 161.
9. Hamre, D., Bernstein, J., and Donovick, R.: Activity of *p*-aminobenzaldehyde, 3-thiosemicarbazone on vaccinia virus in the chick embryo and in the mouse. Proc. Soc. exp. Biol., N. Y., 1950, *73*, 275.
10. Hamre, D., Brownlee, K. A., and Donovick, R.: Studies on the chemotherapy of vaccinia virus. II. The activity of some thiosemicarbazones. J. Immunol., 1951, *67*, 305.
11. Hoagland, C. L., Lavin, G. I., Smadel, J. E., and Rivers, T. M.: Constituents of elementary bodies of vaccinia. II. Properties of nucleic acid obtained from vaccine virus. J. exp. Med., 1940, *72*, 139.
12. Horsfall, F. L., Jr.: Experiments on chemical alteration of virus infections. Harvey Lect., Balt., 1952-1953, Series *XLVIII*, 35.
13. Horsfall, F. L., Jr.: Approaches to the chemotherapy of viral diseases. Bull. N. Y. Acad. Med., 1955, *31*, 783.
14. Horsfall, F. L., Jr. and McCarthy, M.: The modifying effects of certain substances of bacterial origin on the course of infection with pneumonia virus of mice (PVM). J. exp. Med., 1947, *85*, 623.
15. Hurst, E. W., Melvin, P., and Peters, J. M.: The prevention of encephalitis due to the viruses of Eastern equine encephalomyelitis and louping-ill: experiments with trypan red, mepacrine, and many other substances. Brit. J. Pharmacol., 1952, *7*, 455.

16. Jungeblut, C. W.: Chemotherapeutic effects of a naphthoquinonimine on infection of mice with Columbia-SK group of viruses. Proc. Soc. exp. Biol., N. Y., 1951, *77,* 176.

17. Knight, C. A.: The chemical constitution of viruses. Advanc. Virus Res., 1954, *2,* 153.

18. Matthews, R. E. F. and Smith, J. D.: The chemotherapy of viruses. Advanc. Virus Res., 1955, *3,* 49.

19. Moore, A. E. and Friend, C.: Effect of 2,6-diaminopurine on the course of Russian Spring-Summer Encephalitis infection in the mouse. Proc. Soc. exp. Biol., N. Y., 1951, *78,* 153.

20. Moulder, J. D. and Weiss, E.: Purification and properties of the agent of feline pneumonitis. J. infect. Dis., 1951, *88,* 56.

21. Nelson, J. B.: Acute hepatitis associated with mouse leukemia. V. The neurotropic properties of the causal virus. J. exp. Med., 1955, *102,* 581.

22. Peters, D. and Stoeckenius, W.: Untersuchungen am Virus der Variola-Vaccine. III. Mitt.: Enzymatischer Abbau des Innenkörpers. Z. Naturforsch., 1954, *9b,* 524.

23. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehn, M. M., and Baker, L. A.: A filtrate with chemoprophylactic and chemotherapeutic action against MM and Semliki Forest viruses in mice. Antibiot. and Chemother., 1952, *2,* 432.

24. Powell, H. M. and Culbertson, C. G.: Action of an antiviral mold filtrate against MEFl poliomyelitis virus in mice. Proc. Soc. exp. Biol., N. Y., 1953, *83,* 161.

25. Schnitzer, R. J., Buck, M., and Steiger, N.: Chemotherapeutic effect of 2-hydroxy-1,4-naphthoquinonimine on infections of mice with Col SK virus. Proc. Soc. exp. Biol., N. Y., 1951, *77,* 182.

26. Shope, R. E.: An antiviral substance from *Penicillium funiculosum.* I. Effect upon infection in mice with swine influenza virus and Columbia SK encephalomyelitis virus. J. exp. Med., 1953, *97,* 601.

27. Shope, R. E.: An antiviral substance from *Penicillium funiculosum.* II. Effect of helenine upon infection in mice with Semliki Forest virus. J. exp. Med., 1953, *97,* 627.

28. Shope, R. E.: An antiviral substance from *Penicillium funiculosum.* III. General properties and characteristics of helenine. J. exp. Med., 1953, *97,* 639.

29. Tamm, I.: Inhibition of influenza and mumps virus multiplication by 4,5,6-(or 5,6,7-)trichloro-1-β-D-ribofuranosylbenzimidazole. Science, 1954, *120,* 847.

30. Tamm, I.: Selective inhibition of virus multiplication by synthetic chemicals. Bull. N. Y. Acad. Med., 1955, *31,* 537.

31. Tamm, I.: Selective chemical inhibition of influenza B virus multiplication. J. Bact., 1956, *72,* 42.

32. Tamm, I., Folkers, K., and Horsfall, F. L., Jr.: Inhibition of influenza virus multiplication by 2,5-dimethylbenzimidazole. Yale J. Biol. Med., 1952, *24,* 559.

33. Tamm, I., Folkers, K., and Horsfall, F. L., Jr.: Inhibition of influenza virus multiplication by alkyl derivatives of benzimidazole. II. Measurement of inhibitory activity by hemagglutination titrations. J. exp. Med., 1953, *98,* 229.

34. Tamm, I., Folkers, K., and Shunk, C. H.: High inhibitory activity of certain halogenated ribofuranosylbenzimidazoles on influenza B virus multiplication. J. Bact., 1956, *72,* 54.

35. Tamm, I., Folkers, K., and Shunk, C. H.: Certain benzimidazoles, benzenes, and ribofuranosylpurines as inhibitors of influenza B virus multiplication. J. Bact., 1956, *72,* 59.

36. Tamm, I., Folkers, K., Shunk, C. H., Heyl, D., and Horsfall, F. L., Jr.: Inhibition of influenza virus multiplication by alkyl derivatives of benzimidazole. III. Relationship between inhibitory activity and chemical structure. J. exp. Med., 1953, *98,* 245.

37. Tamm, I., Folkers, K., Shunk, C. H., and Horsfall, F. L., Jr.: Inhibition of influenza virus multiplication by N-glycosides of benzimidazoles. J. exp. Med., 1954, *99*, 227.

38. Tamm, I. and Overman, J. R.: Relationship between structure of benzimidazole derivatives and inhibitory activity on vaccinia virus multiplication. In manuscript.

39. Thompson, R. L., Davis, J., Russell, P. B., and Hitchings, G. H.: Effect of alipathic oxime and isatin thiosemicarbazones on vaccinia infection in the mouse and in the rabbit. Proc. Soc. exp. Biol., N. Y., 1953, *84*, 496.

40. Thompson, R. L., Minton, S. A., Jr., Officer, J. E., and Hitchings, G. H.: Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse. J. Immunol., 1953, *70*, 229.

41. Thompson, R. L., Price, M. L., and Minton, S. A., Jr.: Protection of mice against vaccinia virus by administration of benzaldehyde thiosemicarbazone. Proc. Soc. exp. Biol., N. Y., 1951, *78*, 11.

42. Thompson, R. L., Price, M., Minton, S. A., Jr., Falco, E. A., and Hitchings, G. H.: Protection of mice against the vaccinia virus by the administration of phenoxythiouracils. J. Immunol., 1951, *67*, 483.

43. Weiss, E.: The nature of the psittacosis-lymphogranuloma group of microorganisms. Ann. Rev. Microbiol., 1955, *9*, 227.

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THE RELATION BETWEEN LYSOGENICITY IN *ESCHERICHIA COLI* AND *BACILLUS MEGATHERIUM* TO THEIR SUSCEPTIBILITIES TO CHLORAMPHENICOL AND TO STREPTOMYCIN†

In studies on antibiotic susceptibility and resistance carried on in our laboratory[10, 12, 13, 14, 15] we have found it advantageous on numerous occasions to employ auxotrophic mutants derived from *Escherichia coli*, strain K 12, since appropriate resistant mutants could be recombined for further analysis of genetic relationships. The demonstration[5, 16] that this strain is often lysogenic for a phage λ has raised the question whether antibiotic susceptibility data on K 12 strains, or indeed lysogenic strains of any bacterium, are quantitatively applicable to the corresponding non-lysogenic stocks. Since lysogenic stocks occur not infrequently in clinical material, particularly among virulent strains of *Corynebacterium diphtheriae*,[5, 6] of certain of the salmonellae,[1] and of staphylococci this problem is of some general interest.

The basis of the question arises from the growing evidence that lysogenicity may significantly affect normal metabolism, and such other microbial characteristics as virulence, toxin production and immunity to certain other phages.[2, 5, 6, 9] Since microbial susceptibility to antibiotics is also closely linked to metabolism it is evident that at least special cases of association between lysogenicity and altered antibiotic susceptibility may arise when common metabolic pathways are utilized. However, since the actual pathways involved in antibiotic action, and perhaps also in lysogenicity, appear to vary with the species of microorganism, extensive trials of various combinations of agents and organisms would have to be made to establish the general outlines of this thesis. As a more modest objective we have investigated only the materials principally employed in our other investigations.

In contrast to these specific effects there is also the possibility that lysogenicity may result in more generalized changes, perhaps in permeability, which could affect the quantitative thresholds toward a larger variety of

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agents, much as has been claimed for certain dwarf G forms of bacteria.[5] However, explicit data to this point, on properly controlled paired materials do not appear to be available and such measurements therefore constituted a second objective of the present study.

As the nonlysogenic reference type for *E. coli*, strain K 12, we have used the variant K 12S, which was originally isolated by Dr. Esther Lederberg following treatment of the parental stock with ultraviolet light.[8, 16] For the comparison stock we have employed a threonine-leucine-B_1-requiring mutant whose antibiotic response had been found to be typical of our K 12 stocks; the lysogenicity of at least 95% of the cells was confirmed by plaque counts after ultraviolet irradiation, with stock S as indicator.[9] The two cultures were titrated by a photometric growth assay[13, 14] against both streptomycin and chloramphenicol; the optical densities noted were converted to percentages of growth and plotted as ordinates on logarithmic-probability paper, against the corresponding drug concentrations.[11] A typical titration is illustrated in Figure 1. Since the points for the two cultures fitted the same line, within the experimental deviation of 5%, no effect of lysogenicity on antibiotic susceptibility could be demonstrated for this system.

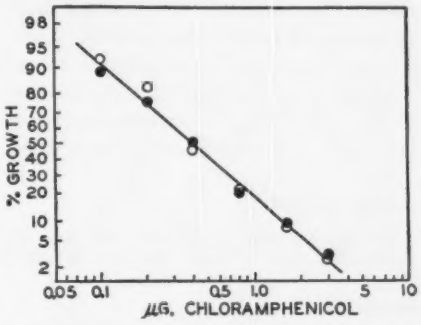

FIG. 1. Photometric growth assay of antibiotic susceptibility. Black circles: nonlysogenic *E. coli* strain K 12 S. Open circles: lysogenic K 12 variant. The special coordinates used are referred to in the text.

A more extensive series of assays was made with a non-lysogenic strain of *B. megatherium* and four derived stocks, each of which was lysogenic for one member of a group of phages which have been designated elsewhere as phages M1, M2, M5 and M7.[4, 7] It is estimated that for two of these stocks at least 99% of the cells were lysogenic; the infection rate for the remaining stocks was considerably lower. Although the titrations for these stocks did not yield as strictly linear response curves as did those for *E. coli*, the points for five replicate titrations all clustered about the same line and no consistent deviations could be demonstrated. It may therefore be concluded that the lysogenic cultures had the same susceptibility to streptomycin as did the nonlysogenic reference stock.

Comment. Although the data presented are limited we have no reason to believe that they are unrepresentative, since the organisms employed are typical of many lysogenic systems, and streptomycin and chloramphenicol are representative antibiotics in photometric assays. From the equal susceptibilities of the nonlysogenic parental stocks and the lysogenic stocks derived from them we may therefore conclude, provisionally, that lysogenicity does not appear to result in any general alteration in antibiotic susceptibility, although as has been noted, specific effects may perhaps occur with certain combinations of agents and organisms. The reason for designating this conclusion as provisional is that, in our present state of knowledge, we cannot be absolutely certain the reference stocks designated as nonlysogenic are, indeed, completely free of phage in latent form. We can only maintain that they are so by the fact that they have, as yet, never been shown to carry virus; perhaps future experimentation may disclose other criteria which may make a re-examination of such systems necessary.

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REFERENCES

1. Boyd, J. S. K.: The symbiotic bacteriophages of *Salmonella typhimurium*. J. Path. Bact., 1950, *62*, 501.
2. Braun, W.: Bacterial dissociation. Bact. Rev., 1947, *11*, 75.
3. Cohen, S. S.: Comparative biochemistry and virology. *Advances in virus research*, III, 1. New York, Academic Press, Inc., 1955.
4. Cowles, P. B.: Lysogenesis in *Bacillus megatherium*. Yale J. Biol. Med., 1953, *25*, 313.
5. Freeman, V. J.: Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. J. Bact., 1951, *61*, 675.
6. Freeman, V. J. and Morse, I. U.: Further observations on the change to virulence of bacteriophage-infected avirulent strains of *Corynebacterium diphtheriae*. J. Bact., 1952, *63*, 407.
7. Friedman, M. and Cowles, P. B.: The bacteriophages of *Bacillus megatherium*. J. Bact., 1953, *66*, 379.
8. Lederberg, E. M. and Lederberg, J.: Genetic studies of lysogenicity in *Escherichia coli*. Genetics, 1953, *38*, 51.
9. Lwoff, A.: Lysogeny. Bact. Rev., 1953, *17*, 269.
10. Muschel, L. H. and Treffers, H. P.: Quantitative studies on the bactericidal actions of serum and complement. I. A photometric growth assay for bactericidal activity. J. Immunol., 1956, *76*, 1.
11. Treffers, H. P.: The linear representation of photometric growth assay data. J. Bact., 1956 (in press).
12. Treffers, H. P. and Alexander, D. C.: The effect of chemical substitution on the activity and resistance patterns of streptomycin derivatives. In: *Antibiotics annual 1955-1956*. New York, Medical Encyclopedia, Inc., 1956, p. 802.
13. Treffers, H. P., Belser, N. O., and Alexander, D. C.: Genetic studies on resistance to streptomycin and to a substituted streptomycin (N'-α-hydroxypropylstrepto-

mycylamine). *Antibiotics annual 1953-54.* New York, Medical Encyclopedia, Inc., 1954, p. 595.

14. Treffers, H. P. and Muschel, L. H.: The combined actions of chloramphenicol and of bactericidal antibody plus complement on *Salmonella typhosa.* J. exp. Med., 1954, *99,* 155.
15. Treffers, H. P., Spinelli, V., and Belser, N. O.: A factor (or mutator gene) influencing mutation rates in *Escherichia coli.* Proc. Nat. Acad. Sc., 1954, *40,* 1064.
16. Weigle, J. J. and Delbrück, M.: Mutual exclusion between an infecting phage and a carried phage. J. Bact., 1951, *62,* 301.

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FREEZING POINT DEPRESSION OF ARTERIAL AND VENOUS PLASMAS *IN VIVO*‡

INTRODUCTION

In an earlier paper[3] we have reported results demonstrating that changing the concentrations of the carbon dioxide and oxygen with which the bloods of sheep and goats are equilibrated has a measurable effect on the total osmotic pressure of the true plasma. At constant oxygenation the osmotic pressure of the true plasma is increased 0.9 milliosmoles/Kg H_2O for each millimole of CO_2/Kg H_2O added to the whole blood. When the carbon dioxide content of the whole blood is held constant, reduction of one millimole of hemoglobin/Kg H_2O, (M.W. 17,000), lowers the osmotic pressure of the true plasma by circa 0.3 milliosmoles/Kg H_2O.

From these data the effect of the exchange of the respiratory gases on the osmotic pressure of the plasma as the blood passes through the pulmonary capillaries can be calculated:

$$\Delta m' = 0.9\,\Delta CO_2 + \Delta 0.3\,O_2 \qquad (1)$$

when $\Delta m'$ represents the arterial venous difference in the osmotic pressure of the true plasma in milliosmoles/Kg H_2O, ΔCO_2 and ΔO_2 the arterial-venous differences in the CO_2 and O_2 contents of the whole blood expressed in millimoles/Kg H_2O.

Since CO_2 and O_2 are respectively given off and taken up in approximately equal amounts by the blood in its passage through the lungs, and since CO_2 exerts per millimole an osmotic effect three times that of O_2, the over-all effect of the exchange must be a reduction in the total osmotic pressure of the plasma as the blood is arterialized in the lungs. For example, when the respiratory quotient is one, each millimole of CO_2 added to the blood per kilogram of water it contains should increase the osmolality by 0.6 milliosmoles per kilogram of water. At 38° C., 0.6 milliosmoles per

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kilogram of water correspond to a pressure of 11.4 mm. Hg. Though this increase in osmotic pressure of 0.6 milliosmoles represents a change of but 0.2% of the total, its absolute value may be of some interest in a discussion of the water exchange between blood and the interstitial fluids. Further, if the production of CO_2 results in an appreciable arterial venous difference in osmotic pressure, as the results of experiments *in vitro* indicate to be the case, the regulation of the elimination of CO_2 must play some rôle in the maintenance of a constant osmotic pressure in the blood.

Considerations such as these prompted us to measure the difference in the osmolality of plasma across the lungs in order to determine directly the effect of the exchange of the respiratory gases on the osmotic pressure of the plasma.

MATERIALS AND METHODS

With the aid of a fluoroscope, a catheter was passed into the right heart of a dog anesthetized with Nembutal that had been heparinized earlier. Twenty cc. samples were drawn simultaneously into well-oiled syringes from the right ventricle and the femoral artery. Ten dogs were so studied. A part of each sample was transferred under oil into tubes and the cells separated by centrifugation at 38° C. The oxygen and carbon dioxide contents of the remainder were determined in the usual way with the Van Slyke-Neill apparatus. The water content of the blood was measured by drying a known volume of blood to constant weight in an oven at 115° C. The freezing point of the plasma was measured with the Fiske osmometer.* The instrument gives readings in terms of milliosmoles/Kg. H_2O. One milliosmole/Kg. H_2O is equivalent to a freezing point depression of 0.001858° C.

RESULTS

The results of our study are summarized in Table 1. Each value in the column head (m'), is the average of about five measurements of osmotic pressure on each plasma sample. In every case the plasma of the blood drawn from the right ventricle had a freezing point lower than that of the arterial plasma. The difference between the two osmolalities ranged from 0.5 to 1.9 milliosmoles/Kg H_2O. In the eight cases in which the oxygen contents of the two bloods was determined as well as the CO_2, the blood took up on the average 1.59 millimoles of oxygen and lost 1.48 millimoles of CO_2/Kg H_2O in its passage through the lungs; at the same time the average decrease in the osmolality of the plasma was 1.2 milliosmoles/Kg H_2O. The decrease in osmolality calculated according to Equation 1 is 0.85 milliosmoles/Kg H_2O. Inasmuch as the values expressed in Equation 1 were

* Built by the Fiske Associates, Inc., 44 Bromfield Street, Boston 8, Massachusetts.

obtained from the results of experiments on sheep blood, there is the possibility that the discrepancy between the calculated and observed values is not due entirely to errors in analysis, but in part to some difference between the hemoglobins of sheep and dogs with respect to the magnitude of the osmotic effect of oxygenation and reduction.

DISCUSSION

Whenever the ion distribution and osmotic balance between plasma and the interstitial fluid are described in terms of a Donnan equilibrium, the

TABLE 1. CO_2 AND O_2 CONCENTRATIONS ARE GIVEN IN MILLIMOLS PER KG OF WATER; THE OSMOTIC PRESSURE IN MILLIOSMOLALITIES

Arterial blood			*Venous blood*			ΔCO_2	ΔO_2	$\Delta m'$	
CO_2	O_2	m'	CO_2	O_2	m'			*measured*	*calculated*
29.8		294.3	31.2		295.3	+1.4		+1.0	
26.8	8.6	299.7	28.6	7.0	301.3	+1.8	—1.6	+1.6	+1.1
32.1	6.5	300.7	33.6	4.1	301.5	+1.5	—2.4	+0.8	+0.6
27.7		297.0	29.2		298.7	+1.5		+1.7	
26.7	5.0	300.2	28.4	3.5	301.3	+1.7	—1.5	+1.6	+1.1
26.4	6.0	301.0	27.4	4.8	301.5	+1.0	—1.2	+0.5	+0.5
29.6	6.8	304.7	31.2	5.9	306.6	+1.6	—0.9	+1.9	+1.2
28.6	8.8	300.4	30.3	6.5	301.0	+1.7	—2.3	+0.6	+0.8
31.5	6.4	300.6	32.9	5.4	302.1	+1.4	—1.0	+1.5	+1.0
30.1	6.4	300.6	31.2	4.6	301.7	+1.1	—1.8	+1.1	+0.5

assumption is made that the freely diffusable solutes exert no osmotic effect other than that which results from their distribution in the presence of the non-diffusable proteins, i.e., that with respect to them, the system is in thermodynamic equilibrium. Since the protein concentration of plasma is higher than that of the interstitial fluid, the osmotic pressure of plasma is considered to be higher than the osmotic pressure of the interstitial fluid by about 15 mm. Hg, a difference in milliosmolality of 0.8. If the milliosmolality of the plasma is 300.0, that of the interstitial fluid should be 300.0 − 0.8 or 299.2. If we assume these values to be representative of the conditions at the venous end of the capillary, the osmolality of the blood at its arterial end must be, according to the results reported above, less by about 1.0 milliosmoles or 299.0 milliosmoles, as opposed to 299.2 which is the value assigned to the interstitial fluid. If these data are illustrative of conditions at the two extremities of a capillary the difference in osmotic pressure due to the difference in protein concentration on the two sides of the capillary mem-

brane is matched or reversed by the lower concentration of carbon dioxide in the arterialized blood. There is the further possibility to be considered, i.e., that in an actively metabolizing tissue the CO_2 concentration in the plasma at the venous end of the capillary may fail to come into equilibrium with that of the interstitial fluid with the result that the osmotic pressure difference due to the proteins is offset at the distal end of the capillary as well.

To evaluate the rôle of the carbon dioxide in the regulation of the osmotic balance of the body fluids as an aspect of the general economy of the organism consider the case of a man weighing say 70 kilograms on an average diet. He produces every 24 hours between 15 and 30 osmoles of CO_2, circa 0.5 of an osmole of urea, whilst ingesting and eliminating 2 kilograms of water and 0.5 of an osmole of salts. These materials, together with others quantitatively less important, are added to and removed from the internal environment which can be represented as a solution of approximately 15 osmoles in 50 Kg of water. Despite the magnitude of the turnover, the osmolality of this internal environment varies but a few milliosmoles per kilogram of water. The standard deviation of the osmolality of arterial blood of male dogs is $\pm$ 2.7 milliosmoles per kilogram of water, as calculated from the data of Table 1, an observation that is in good agreement with the data of Margaria,[1] who measured the osmolality of blood in men and women by means of Hill's thermopile. The loss of water is a continuous process, whereas the intake is discontinuous, accordingly, small fluctuations in osmotic pressure of the fluid of the internal environment are to be expected.

As CO_2 is quantitatively the most important item in the daily osmotic balance, the constancy of the osmotic pressure of the body fluids must depend upon the proper regulation of the elimination of this gas; this regulation of the rate of elimination appears to be primarily directed toward the maintenance of the constant pH of the blood. The production of CO_2 by the tissue cells tends to upset both the hydrogen ion concentration and the osmolality of the internal environment but, so far as the evidence goes, its rate of elimination is regulated through receptors that meter the pH and not through osmoreceptors. The regulation of the hydrogen ion concentration appears to have a priority over that of osmotic pressure in the determination of the rate of elimination of CO_2; the maintenance of a constant osmotic pressure appears to be a by-product of the regulation of pH.

However, the mechanisms regulating pH also serve the purpose of osmotic regulation. Three circumstances may be cited: (i) As blood passes through the tissues, oxygen is released and CO_2 taken up. This uptake of

CO_2 tends to increase both the osmolality and the hydrogen ion concentration of the blood, whereas the simultaneous reduction of the blood tends to buffer both the pH and the osmolality. If the oxygenation and reduction of the hemoglobin were without effect on the osmolality of the blood, the addition of 1 millimole of CO_2 to 1 kilogram of water of the blood would increase the osmolality 0.9 milliosmoles/Kg H_2O. The actual change, to the contrary, according to equation 1, for an R.Q. of 0.86 will be

$$0.9 - .3/0.86 \text{ or } 0.55 \text{ milliosmoles/Kg } H_2O$$

(ii) Whenever organic acids, such as lactic or keto acids, (A^-), enter the blood, both its pH and osmolality tend to be disturbed, whilst the reaction

$$HA + Na^+ + HCO_3^- \rightarrow Na^+ + A^- + H_2O + CO_2$$

with the elimination of CO_2 through the lungs tends to restore both toward their original value. (iii) When the alkali reserve of the plasma drops, the kidneys react by excreting ammonium chloride, thus increasing the (HCO_3^-) (Cl^-) ratio leaving the sum $(HCO_3^-) + (Cl^-)$ unchanged, i.e., the alkali reserve is increased but the osmolality is held constant.

Since the rate of elimination of CO_2 through the lungs is regulated through the respiratory system, the rôle of that system in the maintenance of the constant osmolality of the blood merits some consideration. Assume for purposes of discussion that the elimination of the main metabolic end product, CO_2, as a gas was not possible and that it was necessary to eliminate it in solution, as is the case for urea. To excrete 15 to 30 osmoles of CO_2 produced each day in isotonic solution would require between 50 to 100 liters of water; if the animal were equipped with organs as competent in performing osmotic work as the kidneys, about 10 liters of water would still be required for the elimination of all of the CO_2 produced in a single day. Thus the ability of the animal to eliminate CO_2 as a gas provides an inexpensive path for the excretion of the major metabolic end product with a loss of as little as 300 ml. of water through evaporation.

The capacity of the kidney to produce a hypertonic urine is generally recognized as a feature of fundamental importance to the over-all economy of the terrestrial animal, for it enables him to conserve water. This conservation is further enhanced in some forms by the elimination of uric acid as the end-product of protein metabolism, which is excreted in a solid form after reabsorption of water from the cloaca. Of at least equal importance in the successful invasion of the land by animals are the physicochemical properties of CO_2 that permit the elimination of the bulk of their metabolic end products with a minimal loss of water.

SUMMARY

The freezing point depression of arterial and venous plasmas across the lungs has been measured in 10 dogs. The osmotic pressure of the arterial plasma is, on the average, 1.2 milliosmols per Kg of water lower than that of the venous plasma. The significance of this finding in relation to the osmotic balance between blood and interstitial fluids is discussed and the rôle of the regulation of CO_2 elimination in the maintenance of a constant osmotic pressure of the internal environment is also considered.

REFERENCES

1. Margaria, R.: The vapour pressure of normal human blood. J. Physiol., 1930, *70*, 417.
2. Meschia, G. and Barron, D. H.: Effect of CO_2 and O_2 content of blood on the freezing point of plasma. Quart. J. exp. Physiol., 1956, *41*, 180.

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SOME APPLICATIONS OF PULMONARY PHYSIOLOGY TO CLINICAL MEDICINE

During the past ten years a number of techniques and theoretical concepts have been developed for the analysis of various aspects of pulmonary function in health and in disease. An understanding of the principles of modern respiratory physiology enables the physician to provide the proper amount of artificial ventilation to the apneic subject and to assess the nature and the extent of the functional impairment of the lung in patients with pulmonary disease. This paper outlines certain principles of respiratory physiology, the theoretical basis for some tests of pulmonary function, and some ways in which pulmonary function may be impaired in patients with pulmonary disease.

Table 1 is a convenient classification of pulmonary functions. The first function of the lungs is to provide an optimum gaseous environment for the exchange of carbon dioxide and of oxygen between the blood and the air in the alveoli. This ventilatory function is dependent upon the integrity of the thoracic and diaphragmatic muscles and upon the patency of the airways. Ventilation must be adequate to maintain the proper concentration of oxygen and of carbon dioxide in the alveoli. Next, oxygen must diffuse from the alveoli into the blood and carbon dioxide from the blood into the alveoli. This diffusion function is dependent upon the size of alveolar-capillary surface area available for gas exchange and upon the thinness of the barrier between the alveolar space and the red blood cells. Finally, the blood flowing through the lung must be diverted to the alveoli which are ventilated and not to non-ventilated areas or through arteriovenous communications. One or all of these three functions may be disturbed in disease.

Assessment of ventilatory function and of the total ventilation required by the apneic subject requires an understanding of the factors which determine the composition of the air in the alveoli. Carbon dioxide is produced in the tissues and diffuses from the venous blood into the alveoli, and oxygen is taken up by the blood in the alveolar capillaries and carried to the tissues where it is consumed. The concentration of oxygen and of carbon dioxide in the alveolar gas is dependent upon the metabolic rate and upon the volume of air brought in and out of the alveoli. Since, as will be explained later,

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precise regulation of the alveolar carbon dioxide tension is of greater importance than is the exact maintenance of a normal alveolar oxygen tension, the factors concerned with the alveolar carbon dioxide tension will be considered first. These factors are illustrated in Figure 1, in which the alveoli are represented as a single large space into which carbon dioxide diffuses continuously and into which fresh air is brought periodically. In reality, the

TABLE 1.

Pulmonary function	*Test*	*Clinical finding*
Ventilation of alveoli	Alveolar gas composition Spirometry A. Maximum breathing capacity B. Vital capacity C. Timed vital capacity	Diminished respiratory excursions Airway obstruction Respiratory acidosis with or without anoxemia.
Diffusion of oxygen from alveoli to blood	Diffusing capacity of lung A. Low oxygen method B. Carbon monoxide method	Anoxemia aggravated by exercise, relieved by oxygen breathing
Distribution of blood and air in lung	Alveolar-arterial oxygen tension difference	
A. Effective right-to-left shunts	Per cent venous admixture	Anoxemia during oxygen breathing
B. Uneven ventilation and perfusion	Lung dead space Lung volumes Mixing of gases in lung	Hyperinflation of lung Anoxemia (relieved by oxygen breathing) with CO_2 retention

alveolar space is subdivided into a large number of very small compartments so that the composition of gases in the entire alveolar space is relatively homogeneous. Since fresh air contains less than .1% carbon dioxide, the concentration of carbon dioxide in the alveoli equals the amount of CO_2 produced per minute divided by the volume of air that enters and leaves the alveoli per minute (the alveolar ventilation). The partial pressure or tension of a gas is equal to the barometric pressure (minus the vapor pressure of water) times the per cent of the gas. These relationships may be expressed as follows: $\dot{V}_A = \frac{\dot{V}_{CO_2} \times (P_B - 47)}{P_{A_{CO_2}}}$ (eq. 1) where $\dot{V}_A$ is alveolar ventilation (litres/minute), $\dot{V}_{CO_2}$ is the amount of carbon dioxide produced per minute and $P_{A_{CO_2}}$ is the alveolar CO_2 tension. Since the $P_{A_{CO_2}}$ should be

and normally is maintained at 40 mm. Hg, the alveolar ventilation is directly dependent upon the metabolic rate (or, in this instance, the CO_2 produced per minute). One can, then, predict the alveolar ventilatory requirements of an apneic patient simply from a knowledge of the minute carbon dioxide production. The latter may be measured directly or may be estimated from normal standards (based on body surface area), corrected for fever or activity. Knowledge of the desired alveolar ventilation is only part of the requirement, because the total ventilation also includes a fraction of air which merely goes in and out of the air-conducting passages, the dead space. If one assumes a normal value for dead space (the best assumption at the present time appears to be to equate the dead space in cubic centimeters to the weight in pounds),[5] one can compute this dead space ventilation by multiplying the volume of the dead space by the respiratory rate. Addition of the dead space ventilation to the alveolar ventilation gives the total ventilatory requirement, and, if this is divided by the respiratory rate, the tidal volume is computed. Thus, assessment of an individual's ventilatory requirements is a relatively precise matter for which certain assumptions are made regarding the relationship between physiological dead space and metabolic rate and body weight. This may be done by direct calculation or by means of a nomogram.[5] For a given dead space, the magnitude of the dead space ventilation increases in direct proportion to an increase of the respiratory rate. Thus, an individual has to breathe a larger total volume at fast rates than at slow rates. An individual with psychogenic hyperventilation may breathe very large total volumes at a fast rate, but, since the dead space ventilation is very large, alveolar ventilation may be increased only slightly or not at all. For managing an apneic patient, one does well to select a slow respiratory rate and a large tidal volume so that the dead space occupies a small proportion of the tidal volume. Then, total ventilatory requirements are as small as possible, and if the dead space is not exactly the assumed value, the effect of the error on alveolar ventilation will be minimal.

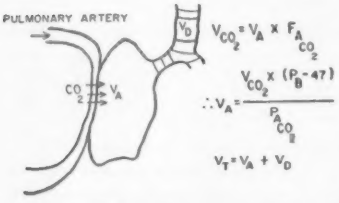


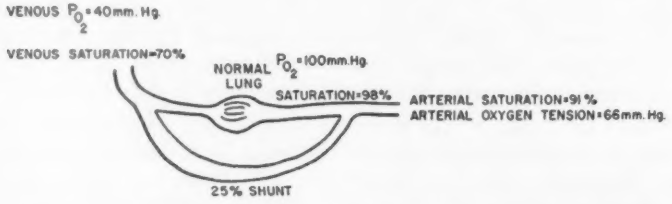
FIG. 1. Schematic representation of relationships between CO_2 production (V_{CO_2}), alveolar ventilation (V_A), alveolar carbon dioxide concentration ($F_{A_{CO_2}}$) and alveolar carbon dioxide tension ($P_{A_{CO_2}}$), during the course of a single expiration. Since the amount of CO_2 added to the alveoli equals the amount of CO_2 ventilated from the alveoli, $V_{CO_2} = V_A \times F_{A_{CO_2}}$, and the volume of air ventilating the alveoli can be expressed as a function of V_{CO_2} and $P_{A_{CO_2}}$. The tidal volume (V_T) equals V_A plus the volume of the dead space (V_D).

If the alveolar carbon dioxide tension ($P_{A_{CO_2}}$) is kept at a normal value, 40 mm. Hg, the alveolar oxygen tension ($P_{A_{O_2}}$) varies from approximately 96-115 mm. Hg, depending upon the respiratory quotient: (eq. 2) $P_{A_{O_2}} = P_{I_{O_2}} - P_{A_{CO_2}} \left(.21 + \frac{.79}{R}\right)$* where $P_{I_{O_2}}$ is the inspired oxygen tension, normally about 148 mm. Hg at sea level. Since the arterial blood is 90% saturated with oxygen when the oxygen tension is 70 mm. Hg, only a marked reduction of alveolar oxygen tension (in the absence of a significant alveolar-arterial oxygen pressure difference) will result in significant anoxemia. Such a lowering of the oxygen tension would result in a marked elevation of the carbon dioxide tension and, indeed, detrimental hypercapnia would develop long before anoxemia. Furthermore, hyperventilation causes elevation of the alveolar oxygen tension which is harmless, whereas it also causes hypocapnia, which may be deleterious.

Proper ventilation of the alveoli is only the first of the three steps involved in the proper oxygenation of the blood. Next, oxygen must diffuse across the alveolar membrane into the capillary blood, and carbon dioxide must diffuse into the alveoli. Since carbon dioxide is approximately 20 times as diffusible as oxygen, impaired diffusion of carbon dioxide is rarely a problem, whereas the diffusion of oxygen may be significantly impaired in disease. The factors concerned in the diffusion of oxygen are best expressed in the formula for calculation of the diffusion capacity of the lung, (D_{O_2}), a measure of the area available for diffusion and of the thinness of the diffusion surface: $D_{O_2} = \frac{\dot{V}_{O_2}}{P_{A_{O_2}} - P\bar{C}_{O_2}}$ (Eq. 3) where $\dot{V}_{O_2}$ equals oxygen consumption per minute, or metabolic rate, and the denominator equals the difference in oxygen tension between the alveoli ($P_{A_{O_2}}$) and the mean oxygen tension of the blood flowing through the alveoli ($P\bar{C}_{O_2}$).[6] This expression is a measure of the oxygen consumed per unit pressure head available for diffusion. Normally, the diffusing capacity is so high that there is no difference between the oxygen tension in the alveoli and that in the blood leaving the alveoli.[4] In patients with the loss of the alveolar-capillary surface (pulmonary emphysema) or with pulmonary fibrosis, the diffusing capacity may be greatly reduced and even result in reduction of the arterial oxygen tension.[3] Usually, this reduction is small until the subject exercises. When the oxygen consumption increases, an appreciable difference of oxy-

* The expression in parenthesis is a correction necessitated by the fact that the number of molecules of CO_2 added to alveolar air does not equal the number of molecules of O_2 removed from inspired air unless the respiratory exchange ratio (R) is 1. The derivation of this equation is explained by Comroe *et al.*[1]

gen tension may exist between the alveoli and the blood leaving them. Characteristically, patients with pulmonary fibrosis show a lowering of their arterial saturation during exercise. Elevation of the pressure of oxygen in the alveoli by the inspiration of pure oxygen greatly increases the pressure head from the alveolus to the blood entering the alveolus. Such an increase of this pressure head results in the rapid diffusion of oxygen into the mixed venous blood so that the blood leaves the alveoli fully saturated with oxygen. Stated differently, for a given diffusing capacity, elevation of the alveolar oxygen tension from 100 to 700 mg. Hg causes oxygen to diffuse



AMOUNT OF O_2 FROM ALVEOLI + AMOUNT OF O_2 FROM SHUNT = AMOUNT OF O_2 IN ARTERY

AMOUNT OF O = % SATURATION X BLOOD FLOW

TOTAL BLOOD FLOW = SHUNT FLOW + (1-SHUNT FLOW) = 1

.98 (1-.25) + .70 (.25) = .91 = ARTERIAL O_2 SATURATION

FIG. 2. Method for calculating volume of right-to-left shunt when end-capillary, venous, and arterial oxygen saturations are known (or assumed).

from the alveoli into the venous blood some eight times as fast as it did during the inspiration of room air, with the result that the blood flowing through the alveolar capillaries soon becomes fully saturated.[7] Clinically, patients with diffusion problems may show a marked increase of arterial oxygen saturation during oxygen breathing. For the same reason, inspiration of a low oxygen mixture, or ascent to high altitude, greatly increases the difference in oxygen tension between the alveoli and the blood leaving the alveoli. An individual whose diffusing capacity is moderately reduced may have a normal oxygen saturation at sea level but may suffer from severe anoxemia at 12-15,000 feet. This effect of hypoxia on increasing the alveolar-arterial oxygen tension difference when due to impaired diffusion is the basis for the low-oxygen method of measuring the diffusing capacity of the lung.

The final pulmonary function to be considered concerns the distribution of blood and of gas in the lung. Obviously, if all of the air went to some alveoli and all of the blood went to others, venous blood would pass through the lungs unchanged. Normally, circulation and ventilation are so related

that the blood flows to the ventilated alveoli. If an area of the lung is diseased, a number of compensatory mechanisms serve to shunt blood away from that area. However, many diseases cause impaired blood-gas distribution which results in cyanosis. Indeed, cyanosis is most commonly a result of impaired distribution. For example, as in Figure 2, a pulmonary arteriovenous fistula results in the admixture of venous blood to the arterialized blood such that the saturation of the normally oxygenated blood is reduced from 98 to 91%. With knowledge of the venous saturation, one can calculate the percentage of blood, (x), shunted around normally ventilated alveoli: $S_{C_{O_2}}$ (1—x) + $S_{V_{O_2}}$ (x) = $S_{a_{O_2}}$ (eq. 4), where $S_{V_{O_2}}$ is the venous oxygen saturation, $S_{C_{O_2}}$ is the oxygen saturation in the blood leaving the ventilated alveoli and $S_{a_{O_2}}$ is the arterial oxygen saturation. Similar shunts may occur through atelectatic lung, or, of course, through cardiac abnormalities, and the same calculation of the per cent of shunt applies. The low oxygen saturation existing in patients with a shunt of this sort is only partially improved by the inspiration of pure oxygen. Oxygen breathing raises the venous saturation and the saturation of the blood leaving the alveoli so that the arterial saturation may rise as much as 10%. Solution of equation 4 during oxygen breathing provides a fairly accurate estimation of the per cent shunt, since other causes of oxygen unsaturation are eliminated by this procedure. Such a calculation is only valid if the shunt is over 25% of the total blood flow, since smaller shunts will not cause appreciable oxygen unsaturation during oxygen breathing. Because of the relatively steep slope of the carbon dioxide dissociation curve of blood, elevation of the arterial carbon dioxide tension occurs only in extensive right-to-left shunts.

Another type of distribution problem, seen particularly in pulmonary emphysema, occurs when different areas of lung are ventilated at different rates.[8] For example, as in Figure 3, if one lung is ventilated twice as much as the other, the alveolar carbon dioxide tension in one lung is 60 mm. Hg, whereas in the other it is 30 mm. Hg. Assuming equal blood flows to the two lungs, the mean CO_2 tension in the blood is the average of that of the blood leaving the two lungs (45 mm. Hg), whereas the average alveolar P_{CO_2} is only 40 mm. Hg, because twice as much air containing a smaller amount of CO_2 contributes to the mean as does air containing the larger amount of CO_2. This type of situation may result in appreciable retention of CO_2 in the arterial blood. Uneven ventilation may also cause significant lowering of the partial pressure of oxygen in the arterial blood. In Figure 3, the alveolar oxygen tension in one lung is 120 mm. Hg and in the other, 60 mm. Hg. The average alveolar tension is 100 mm. Hg. The bloods leaving the two lungs have oxygen tensions of 60 and 120 mm. Hg and

saturations of 90 and 100% respectively. Thus, the arterial oxygen saturation is 95% and the arterial oxygen tension is 80 mm. Hg, an alveolar-arterial tension difference of 20 mm. Hg. This form of distribution impairment only results in a pronounced lowering of the arterial oxygen tension when air is breathed since its effect on arterial oxygen tension is largely dependent on the changing slope of the oxygen dissociation curve and can

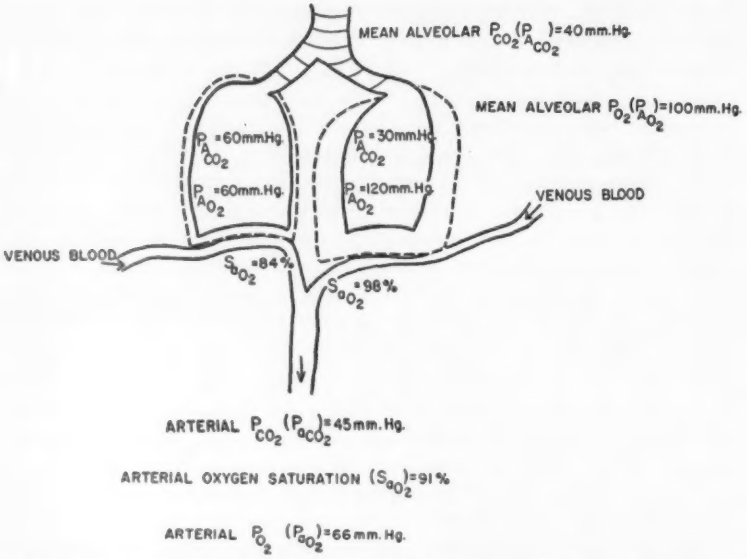


FIG. 3. The mechanism whereby different rates of ventilation to the two lungs result in relative CO_2 retention and anoxemia.

be eliminated by the inspiration of a high or a very low oxygen mixture. A pronounced increase of oxygen saturation during the inspiration of pure oxygen suggests the presence either of impaired diffusion or of uneven ventilation of the lung. Low oxygen saturation during the inspiration of pure oxygen is diagnostic of a shunt or a shunt-like effect in the lung.

Normal respiratory function, then, consists of ventilation of the alveoli with fresh air in sufficient quantity to dilute the carbon dioxide produced per minute to the proper value (40 mm. Hg), diffusion of oxygen and carbon dioxide across the alveolar membrane and even, homogenous distribution of blood and gas flows in the lung. Since the mechanical act of ventilation is dependent upon the integrity of the thoracic muscles and diaphragm

and the patency of the airways, tests of ventilatory function (maximum breathing capacity, vital capacity, and timed vital capacity) shed light on an individual's ability to ventilate his alveoli. The over-all effectiveness of ventilation can be assessed by measuring the alveolar CO_2 and O_2 tensions. Since ventilatory needs are directly dependent, as has been developed, upon metabolic requirements, an individual with mild ventilatory insufficiency may have normal alveolar gas composition at rest, but a low oxygen tension and a high CO_2 tension during exercise. It is important to bear in mind that ventilatory insufficiency, as opposed to impairment of the other pulmonary functions, results primarily in CO_2 retention and, only in advanced cases, in anoxemia.

Precise measurement of the oxygen diffusing capacity of the lung is done, at the present time, by techniques which are difficult to perform and which require special equipment. Evaluation of the diffusing capacity of the lung is important for assessing cyanosis in patients without evidence of ventilatory dysfunction or of shunts, and clinical signs of impaired diffusion are a drop of arterial oxygen saturation during exercise (in the absence of severe emphysema) and a marked rise of oxygen saturation during the inspiration of pure oxygen.

A number of techniques have been developed to assess homogeneity of ventilation and of perfusion. Measurement of the tension of oxygen in alveolar air and arterial blood allows one to calculate the per cent shunt through unventilated lung or through a right-to-left shunt. If the arterial oxygen saturation is less than 100% during the inspiration of oxygen, one can estimate the per cent shunt, assuming a normal venous saturation and a saturation of 110% (which includes dissolved oxygen) of the blood leaving normally ventilated alveoli. Homogeneity of ventilation is assessed by analysis of expired nitrogen or carbon dioxide curves with rapid gas analyzers, measurement of the size of the lung dead space, and of the lung volumes, since increased dead space and residual volume generally indicate the presence of uneven distribution of inspired air.

With the aid of a few equations or a nomogram the physician is able to predict the ventilatory requirements of the apneic subject with a fair degree of accuracy. These techniques are particularly useful to the internist concerned with the management of respirator patients and to the anesthesiologist who is providing the ventilation for an open-chest patient. An understanding of respiratory physiology and a few simple measurements (involving a spirometer and some technique for measurement of the saturation of the arterial blood) enable the physician to assess ventilatory function, the presence or absence of severe impairment of diffusion of

oxygen in the lung, and the presence or absence of marked impairment of blood and gas distribution in the lung.

REFERENCES

1. Comroe, J. H., Jr., Foster, R. E., II, DuBois, A. B., Briscoe, W. A., and Carlsen, E.: *The lung.* Year Book Publishers, 1955.
2. Donald, K. W., Renzetti, A., Riley, R. L., and Cournand, A.: Analysis of factors affecting oxygen and carbon dioxide in gas and blood of lungs: Results. J. appl. Physiol., 1952, *4,* 497.
3. Farhi, L. E. and Rahn, H.: A theoretical analysis of the alveolar-arterial O_2 difference with special reference to the distribution effect. J. appl. Physiol., 1955, *7,* 690.
4. Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E.: An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude. Amer. J. Physiol., 1946, *147,* 199.
5. Radford, E. P., Jr., Fernis, B. G., Jr., and Kriete, B. C.: Clinical use of a nomogram to estimate proper ventilation during artificial respiration. N. Engl. J. Med., 1954, *251,* 877.
6. Riley, R. L. and Cournand, A.: Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: Theory. J. appl. Physiol., 1951, *4,* 77.
7. Williams, M. H., Jr.: Mechanical versus reflex effects of diffuse pulmonary embolism in anesthetized dogs. Circ. Res., 1956, *4,* 325.

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THE DETERMINATION OF CHROMOSOMAL SEX BY ORAL SMEARS‡

The sex of an individual may be defined in three different ways:

a. The type of gonad present
b. General anatomical appearance and psychological orientation
c. Genetic factors as determined by the presence of the XX or XY chromosomes.

If uniformity is absent or equivocal in these characteristics, the decision as to whether an individual should be classified as male or female is difficult and is usually based on the prevailing general anatomical appearance. This may be unsatisfactory and is often misleading. General anatomical appearance and psychological orientation may be susceptible to spontaneous or iatrogenic changes. The chromosomal sex is perhaps closest to the "true" sex and is of greatest importance in the early recognition and treatment of intersexuality.

As early as 1917 Weiman,[20] and later Painter,[15] showed that sex chromosomes in the developing human male germ cell tend to remain compact with deep staining properties during the nuclear interphase. This is in contrast to other chromosomes which become so diffuse that it is difficult to identify them. Further, Geitler[7] demonstrated a sex difference in nuclear morphology in the *Gerris lateralis* and *Gerris lacustris,* waterbugs, on the basis of heterochromatic properties of their sex chromosomes. The early investigators in this field have therefore speculated that the fusion of the heterochromatic portions of the two X chromosomes in the female would form a mass large enough to be detected by ordinary microscopic means, the small size of the Y chromosome being probably the reason for the minute size of the sex chromatin in male nuclei. It was Barr *et al.* in 1950[2, 8, 18] who described for the first time an intranuclear body in the nerve cell of the cat, identified as the female sex chromatin. Large neurons seemed to be best suitable for this work because their nucleus is vesicular and lacks other

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chromatin particles. In these cells a chromatin mass about one micron in diameter was seen next to the nucleolus. This mass was encountered frequently in female cats but seldom occurred in the male.

The viscosity of the sex chromatin is such that, if free, it takes a round shape, while if adjacent to other structures, it is molded along the surface. If the nucleolus is eccentric, the chromatin is of discoid shape between the nucleolus and the membrane (Fig. 1), lying generally in the vicinity of the nucleolus. The staining qualities are those of desoxyribonucleic acid.

In 1953 Moore, Graham, and Barr[14, 1] devised a technique by which a chromosomal sex determination could be carried out from a skin biopsy in the human. Skin samples were stained by the Harris-Lena method (Gottenberg modification), and 100 cells were counted in the prickle cell layer and checked for the female chromatin. The incidence of this chromatin mass in cells of the skin originating from females was above 60 per cent, while specimens from males showed from 1 to 14 per cent incidence, a significant difference. Other authors have confirmed these findings.[8, 10, 11] Studies on the sex chromatin in other tissues have been published since. Davidson and Smith[5] reported on chromosomal sex determination in neutrophiles from the peripheral blood. Sohval and Gaines[16] have studied the sexual differences in nuclear morphology of tumors, inflammations, and squamous metaplasia. They found no discrepancy in the chromosomal sex of host and tumor except in the instance of a teratomatous growth (see also Cruikshank[4]). Shettles[17] was able to predict fetal sex from squamous cells recovered from amniotic fluid during labor. Moore and Barr,[13] as well as Marberger,[10] have achieved satisfactory results using a mucosal smear stained with a modified Feulgen or cresyl violet stain.

The main application of this new diagnostic tool is in the study of the true and pseudohermaphrodite, especially in early age when hormone studies are difficult to obtain or are not conclusive.

At six to eight weeks in the human embryo the genitalia differentiate from a Wolffian or Mullerian duct system, both of which are present in early embryonic life. It seems to be well established by the work of Jost, Raynard, and Wells that the stimuli for differentiation of the genitalia are provided by the gonads. These authors were able to destroy *in utero* the gonadal anlage before differentiation of the gonad in mice, rats, and rabbits. The fetuses all showed development of the female external genitalia only. The investigators therefore believe that the male primary sex characteristics depend upon stimulation from the gonad. This theory may partially explain why presumably female patients with ovarian agenesis (or better, gonadal dysgenesis) have been reported as chromosomal males.[3, 9, 19, 20] The fact that approximately 80 per cent instead of 50 per cent of these lack the

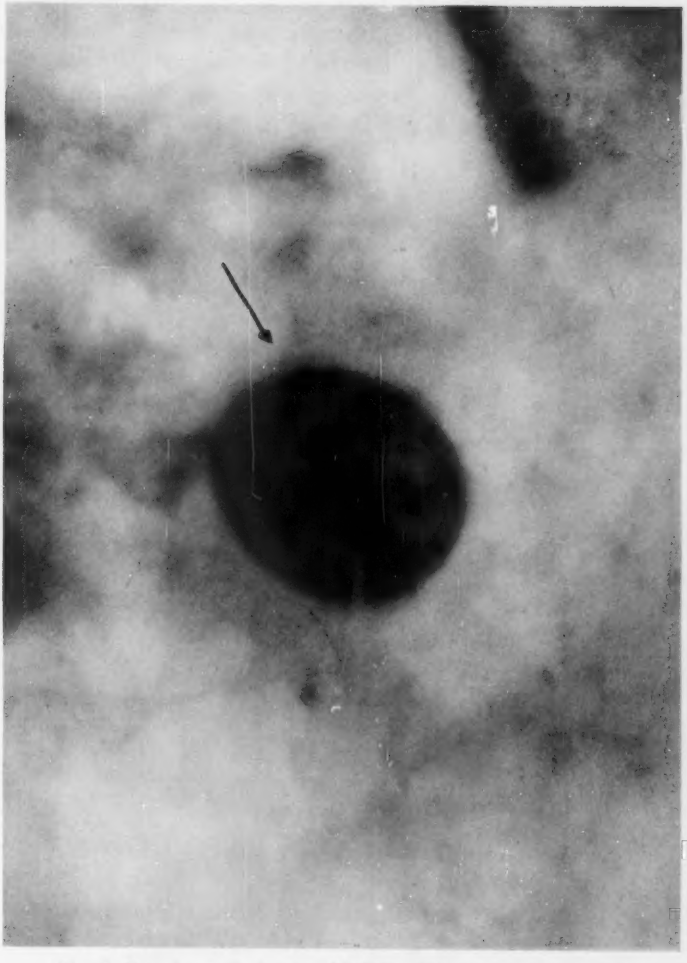

FIG. 1. Sex chromatin in an epithelial cell from the oral mucosa.

female sex chromatin is as yet unexplained. The cause of gonadal agenesis in these cases has not been explained either, but may possibly be attributed to chromosomal aberration or damage.

While the skin biopsy gives very satisfactory results, a simpler and less traumatic method seemed to be desirable. We have therefore analyzed some

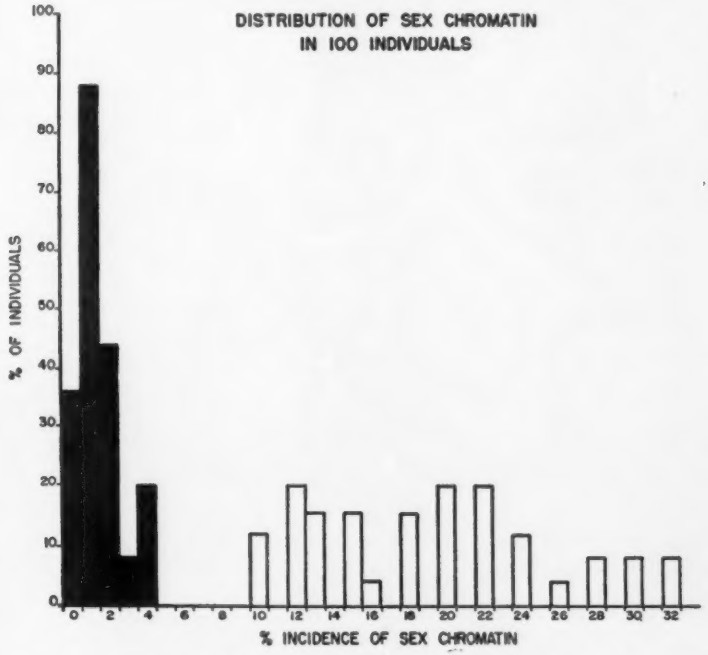

FIG. 2. Distribution of sex chromatin in 100 individuals.

of our routine vaginal smears and found that they also contained the sex chromatin. Oral smears were then done on 100 persons of varying ages for a control series. Fifty males and 50 females each had the buccal mucosa scraped with a wooden spatula. The material obtained was smeared on a glass slide and immediately dropped in a solution containing equal parts of ether and 95 per cent ethyl alcohol. The slides were then stained with the Papanicolaou stain. This stain was used because it had been determined previously that it was satisfactory for identification of the sex component. Each slide was studied under oil immersion lens, and the sex chromatin

was easily identified. The sex of the person from whom the specimen was obtained was not known until after the counts had been made. One hundred cells of the parabasal and precornified type were counted and the percentage of cells containing this component determined. In the control series proper

TABLE 1.

Clinical diagnosis	*Anatomical sex*	*Incidence of female sex chromatin*	*Chromosomal sex*
Pseudohermaphrodite (Adrenal hyperplasia)	female	15%	female
Pseudohermaphrodite (?Adrenal hyperplasia)	female	12%	female
Pseudohermaphrodite (?Adrenal hyperplasia)	female	22%	female
Pseudohermaphrodite (Adrenal hyperplasia)	male	14%	female
Gonadal dysgenesis	female	1%	male
Gonadal dysgenesis	female	1%	male
Gonadal dysgenesis	female	10%	female
Gonadal dysgenesis	female	11%	female
Gonadal dysgenesis	female	10%	female
"Behaviour problem"	male	1%	male
True hermaphrodite	female	34%	female
Primary amenorrhea	female	16%	female
Absence of vagina	female	16%	female
Testicular agenesis	male	4%	male
Multiple congenital malformations	female	48%	female

identification of the sex of the individual was made on the first count on all 100 slides.

As illustrated by Figure 2, there were two definite groups, one containing from 0-4 per cent and the other from 10-32 per cent incidence of sex chromatin respectively. The first group included all the males, while all of the females studied were found in the second group. Furthermore, 84 per cent of the males had an incidence of the sex chromatin of 0-2 per cent, while the females of this control group were rather uniformly distributed between 10 and 32 per cent.

Since this test for sex differentiation has proved so accurate, a decision was made to apply it to those cases where clinical evaluation of sex was difficult or questionable. Fifteen patients of this type have been examined so far with interesting and sometimes unexpected results.

As will be seen from Table 1, four of these cases were clinically diagnosed as pseudohermaphrodites, three being considered anatomically female and one anatomically male. One of the three females was a proven case of congenital adrenal hyperplasia, diagnosis confirmed by the elevated beta fraction of the 17-ketosteroids. The other two were newborn twins on whom no endocrine essays have yet been performed. These twins showed a phallus-like development of the clitoris. All three cases had a female chromosomal pattern. The anatomically male pseudohermaphrodite was 44 years old and had been previously diagnosed as a male achondroplastic dwarf. He had a phallus measuring 3½ inches with hypospadias and a bifid scrotum. No testes were palpable. Steroid studies proved this case also to be a congenital adrenal hyperplasia. A chromatin count of 14 per cent indicated that this patient was genetically female.

Two of the gonadal dysgenesis cases proved to be chromosomally male even though they had female genitalia and were living satisfactory lives emotionally, physically, and mentally as females. This is in accord with the findings of Wilkins[21] and others. The true hermaphrodite had female external genitalia, a testis on one side and an ovary on the other, the chromatin count followed a female pattern. In the remaining cases the chromosomal sex corresponded with the anatomical sex.

In addition to its potentialities in genetic investigation this test has a very real clinical value. This is especially so in assisting the clinically sexually indistinguishable individual toward proper sexual orientation.

CONCLUSIONS

1. A variation of the Barr technique of chromosomal sex differentiation is reported.
2. Its accuracy has been verified on 100 control cases.
3. Its use in problems of clinical sex differentiation is discussed, 15 such cases being reported.

ACKNOWLEDGMENT

The authors wish to express their grateful appreciation to Dr. John McLean Morris for his great interest and encouragement in this work.

REFERENCES

1. Barr, M. L.: An interim note on the application of the skin biopsy test of chromosomal sex to hermaphrodites. Surg., Gynec. Obstet., 1954, *99*, 184.

2. Barr, M. L., Bertram, L. F., and Lindsay, H. A.: The morpholgoy of the nerve cell nucleus according to sex. Anat. Rec., 1950, *107,* 283.
3. Briggs, D. K. and Kupperman, H. S.: Chromatin patterns of polymorphonuclear leukocytes in discrimination of chromosomal sex. Program of the Third Annual Meeting of the Inter-Society Cytology Council, 1955.
4. Cruickshank, S. B.: Sex of mediastinal teratoma. Lancet, 1955, *1,* 253.
5. Davidson, W. M. and Smith, D. R.: Chromosomal sex determination in neutrophiles from the peripheral blood smear. Brit. med. J., 1954, *2,* 3.
6. Emery, J. L. and MacMillan, M.: Observation on the female sex chromatin in human epidermis and on the value of skin biopsy determining sex. J. Path. Bact., 1954, *68,* 17.
7. Geitler, L.: Studium des Kernbaus und der Kernteilung der Wasserlaufer Gerris lateralis und Gerris lacustris. Z. Zellforsch., 1937, *26,* 641.
8. Graham, M. A.: Sex chromatin in cell nuclei of the cat from early embryo to maturity. Anat. Rec., 1954, *119,* 469.
9. Grumbach, M. M., VanWyck, J. W., and Wilkins, H.: Chromosal sex in gonadal dysgenesis. J. clin. Endocr., 1955, *15,* 1161-1193.
10. Marberger, E., Boccabella, R., and Nelson, W. O.: Oral smears as a method of chromosomal sex determination. Proc. Soc. exp. Biol., N.Y., 1955, *89,* 488.
11. Marberger, E. and Nelson, W. O.: Sexual differences in epidermal nuclei of human skin. Anat. Rec., 1954, *118,* 399.
12. Moore, K. L. and Barr, M. L.: Smears from the oral mucosa in the detection of chromosomal sex. Lancet, 1955, *2,* 57.
13. Moore, K. L. and Barr, M. L.: Morphology of nerve cell. J. comp. Neurol., 1953, *98,* 213.
14. Moore, K. L., Graham, M. A., and Barr ,M. L.: The detection of chromosomal sex in hermaphrodites from a skin biopsy. Surg., Gynec., Obstet., 1953, *96,* 641.
15. Painter, T. S.: The sex chromosomes of men. Amer. Natur., 1924, *58,* 506.
16. Polani, P. E., Hunter, W. F., and Lennox, B.: Chromosomal sex in Turner's syndrome with coarctation of the aorta. Lancet, 1954, *2,* 120.
17. Shettles, L. B.: Sex of infant in relation to nuclear morphology of cells in human. Fed. Proc., 1956, *15,* 170.
18. Sohval, A. R. and Gaines, J. A.: Sexual differences in nuclear morphology of tumors, inflammation, hyperplasia and squamous hyperplasia. Cancer, 1955, *8,* 896.
19. Sun, L. C. Y. and Rakoff, A. E.: The determination of chromosomal sex by the peripheral smear method with particular reference to intersex syndromes. Inter-Society Cytology Council, Cleveland, Ohio, 1955.
20. Weiman, H. L.: The chromosomes of human spermatocyles. Amer. J. Anat., 1917, *21,* 1-22.
21. Wilkins, L., Grumbach, M. M., and VanWyck, J. S.: Chromosomal sex in ovarian agenensis. J. clin. Endocr., 1954, *14,* 1270.

BOOK REVIEWS

AN OUTLINE OF THE CYTOLOGY AND PATHOLOGY OF THE RETICULAR TISSUE. By A. H. E. Marshall. With a chapter on the Lipidoses by J. V. Cavanagh. Edinburgh and London, Oliver and Boyd, 1956. x, 274 pp. 43s.

This book in many ways succeeds in being more than the "outline" that it avowedly sets out to be. The concept of "the reticulo-endothelial system" is redrawn. Less is made of the dye-binding and phagocytic properties that have been stressed by Aschoff and Kiyono. These are considered simply a phase in the activity of certain of the cells. The reticular tissue is defined as a "tissue composed of fixed cells (primitive reticular cells) supported on a framework of fibrils and including all cells in the body derived from this tissue." In view of the transformation *in vitro* of monocytes obtained from the blood into fibroblasts as demonstrated by Ebeling, this definition is somewhat unsharp, but it is perhaps as good as any available. The "metalophil" properties of certain elements of the reticular tissue that have been extensively studied by the author are well described. The remarkably large and complex processes of these cells when impregnated with silver are well demonstrated. Correlations of this property with other features of these elements in health and disease require further elaboration.

The book is especially valuable for its lucid presentation of a vast amount of information regarding the form and functions of the reticular tissue in the normal and hyperplastic states. The reactions to hormonal and other influences are well detailed. There is also a timely and important summary of the rôle of these cells in antibody production, and of the anatomical expression of this process and of sensitivity reactions. Results of such recent studies as those with fluorescent antibodies are included.

Less illuminating is the discussion of neoplasia. It requires more justification than is given to apply such perplexing terms as "lympho-reticular medullary reticulosis." Many readers will be surprised to find a firm denial of the transformation of one histological type of reticular neoplasm into another, and that Hodgkin's disease is classified as a benign multi-focal tumor. In this subject, however, each man to his taste for the present.

The section on lipid storage diseases by J. B. Cavanagh is concise, well written, and highly informative. The essential unity of eosinophilic granuloma, Hand-Schüller-Christian disease, and Letterer-Siwe disease is accepted with no perceptible qualms. It is surprising not to find S. B. Wolbach first and foremost among those credited with the interpretation of "sclerosing hemangioma" for the lesion of the skin considered "histiocytoma" by others.

Technically, the book is a credit to the publishers, as well as to the authors. The photomicrographs are abundant, of well-selected fields, and are excellently reproduced. A more careful checking of proper names quoted in the text would have been in better keeping with the other merits of the volume.

This short book meets a real current need. It is hoped that succeeding editions will be forthcoming in as good step with unfolding knowledge, on the same wide front, and with the same mature philosophy and critical sense.

AVERILL A. LIEBOW

PRINCIPLES OF PSYCHOANALYSIS: THEIR APPLICATION TO THE NEUROSES. By Herman Nunberg. New York, International University Press, Inc., 1955. 382 pp. $7.50.

A psychoanalyst with the years of experience of Dr. Nunberg who writes a book concerning his field can hardly fail to turn out something of value and interest. With certain qualifications, this expectation is fulfilled here. The author is obviously an acute and thoughtful observer of human behavior. It is probable that the appropriate group of readers to which this book might be addressed is one with a fair acquaintance with psychoanalytic literature, especially the writings of Sigmund Freud. Of such a group, those who are engaged in the complexities of treating mental illness will appreciate the liberal sprinkling of pungent and useful clinical anecdotes and aphorisms. The chapters on "Anxiety" and on "The Processes of Defense" are especially rich in such findings and in themselves constitute interesting summaries of psychoanalytic theories of the topics mentioned.

Even those for whom such parts of the book will be rewarding must prepare themselves for certain hazards. For example, a great burden is put on the reader to recall previous statements of the author which had not been particularly emphasized when first made. The style of writing is highly assertive, even approaching hyperbole at times. Occasionally, the author seems to equate having discussed an idea with having proven it. The tone is almost always dogmatic, including places where legitimate argument is not only possible, but actually being waged elsewhere in the literature. For instance, this reviewer was paradoxically confused by the high degree of confidence with which the author propounded his views on the murky problem of the "actual neuroses." These various tendencies detract from the total value, although the informed reader need not be seriously offended.

The book cannot be recommended to those newly seeking knowledge of psychoanalysis. The literary style cloaking the ideas may, for such readers, be impenetrable and lead either to rejection of the ideas or a spuriously confident acceptance.

The table of contents suggests a much more highly organized book than it is. The index is good and provides access to many of the clinical tidbits. The "Bibliography" appears to be instead a "Selected Reading List," since the body of the book rarely refers to the writings of others in a clearly identifiable way.

JOHN HIGGINS

HYPNOTIC SUGGESTION—ITS RÔLE IN PSYCHONEUROTIC AND PSYCHOSOMATIC DISORDERS. By S. J. Van Pelt. New York, Philosophical Library, 1956. 95 pp. $2.75.

In the last few years there has been a renewed interest in hypnosis. Unfortunately one can only view this renaissance with mixed feelings. A number of thoughtful clinical, experimental, and theoretical studies have appeared, but the bulk of the writing has either been sensational or uncritical. It is, alas, so easy for a person interested in hypnosis to become at first fascinated and then hypnotized by his labors.

There is little that is new in Dr. Van Pelt's book. There is much, however, with which one can take issue in his historical and theoretical sections. It is surely not true that Freud gave up hypnotism "when he discovered that he could not deeply hypnotize all his patients." This is merely emphasizing one of Freud's less important considerations. There are other and much more important reasons for this turning away from cathartic hypnotherapy, and Freud wrote repeatedly about this.

Since the book is concerned with therapy and a considerable portion of it is devoted to this topic, this reviewer would like to limit his discussion to this aspect. Dr. Van Pelt presents twelve patients, all of whom were cured by hypnotherapy; there were no failures. It is interesting to note that nine of the twelve patients were cured in six sessions, and one could easily speculate on the compliance of the patients in order to make success not only so thorough but also so uniform.

Treatment, choosing one case at random, eventually led to something like this "It was explained to the patient in hypnosis that now he knew the *cause* of his trouble, things would be different. He was no longer a young man who wanted to specialize. He could make a comfortable living as a general practitioner if he pulled himself together. Many people had stood by him all these years and helped him, they would help him more if he made the effort. He no longer regarded his wife as a 'ball and chain.' She had stood by him loyally all these years. He was proud of his son—he did not want him to be ashamed of his father . . . etc." Despite the fact that the patient supposedly knew "the *cause* of his trouble" he had to be told all this and more. After he had thus been successfully brain-washed, he was sent on his way.

It seems to this writer that such suggestions are not only potentially harmful to the patient, but also represent a resurgence of an anti-humanistic, personality-suppressive attitude that cannot be too much deplored in any form of treatment and above all in psychotherapy. One reviewer recently pointed out that it was one of Freud's greatest contributions to have demanded that one should listen attentively to one's patients.

There is much that we have to learn about hypnosis. However, there is much that we already do know, as is so well exemplified by the studies of Brenman and Gill; there is an unsurpassable gulf between their studies and the one reported here. This book increases this writer's conviction about

the need for more carefully controlled and intensive, rather than extensive, research both in clinical and experimental hypnosis.

JAY KATZ

FIFTH ANNUAL REPORT ON STRESS, 1955-56. By Hans Selye and Gunnar Heuser. New York, MD Publications, Inc., 1956. 815 pp.

This current edition of the annual review of new developments in the field of "stress" follows the pattern of the previous ones. The first hundred pages are occupied by a summary of the concept of "stress" as developed by Selye and his colleagues. A great deal of this repeats material from previous editions, some of it word for word. A series of nine review articles is then inserted. These include a discussion of primary aldosteronism by Conn, the effects of hormones on inflammation by Dougherty, stress and the catechol hormones by von Euler, Seymour Gray on adrenal and gastric secretion, Dwight Ingle on the etiology of disease, Don Nelson on factors effecting adrenocortical secretion, Ernst Scharrer on neurosecretion, Spitz on psychiatric stress in infancy, and Stoerk on the lymphoid tissue, cortisone, and immunity. These authors are qualified and their reviews are interesting, although for the most part they don't add previously unpublished material. The remainder of the book, a total of 550 pages, is devoted to a bibliography arranged both in subject and author sequence. In addition to the bibliography, there is an index to the bibliography and a short handbook for interpreting the symbols which are used in bibliographic notation. The *Fifth Annual Report of Stress,* like its predecessors, will be of value to those who accept the basic tenets of Selye's approach to physiology, and the review section will also be of interest to anyone seeking an authoritative résumé of recent advances in the areas under consideration.

PHILIP K. BONDY

THE INQUISITIVE PHYSICIAN. THE LIFE AND TIMES OF GEORGE FRANCIS MINOT. By Francis M. Rackemann. Cambridge, Mass., Harvard University Press, 1956. 288 pp. $5.00.

This interesting biography gives a splendid picture of a unique character and great scientist. The author has brought out in a striking way how Dr. Minot's curiosity constantly led him on into new paths. In addition to this graphic account of Dr. Minot, the author has pointed out the great changes that have taken place in American medicine during the past twenty-five years. He emphasizes the important rôle that Dr. Edsall played in the development of research at Harvard, but in his enthusiasm for his subject does not give adequate credit to the important part the Johns Hopkins Medical School played during these years; this is splendidly brought out by Shryock in his book about the influence of Johns Hopkins on American medicine.

In addition to outstanding contributions of Dr. Minot, the book includes a fascinating picture of his interest in the many young men he trained and

stimulated. It well illustrates how one individual may combine the great qualities of teacher, investigator, and wise councilor.

ERNEST SACHS

MENTAL HEALTH PLANNING FOR SOCIAL ACTION. By George S. Stevenson. New York, The Blakiston Division, McGraw-Hill Book Company, Inc., 1956. 358 pp. $6.50.

Few, if any, have done as much to advance the cause of mental health as the author of this book for which so many have waited with keen anticipation. Dr. Stevenson, with rare insight and judgment, has presented the challenge that "there is more scientific knowledge and technique for dealing with mental illness than is now being used." Incidentally, he observes that, in spite of some strong encouragement from within the field of public health, especially at Yale and Johns Hopkins and at several of the larger foundations, public health officials have been slow to take the initiative in developing the potentialities for mental hygiene. Even though schools of public health have added mental health to their curricula, the author notes that it is too often not sufficiently designed to develop skill in meeting the everyday tasks of public health but is simply a set of lectures in psychiatry as given in medical schools.

Dr. Stevenson examines the mental hospital and makes constructive suggestions about how it can increase its contribution to mental health. He gives test questions for a hospital's success, tells why the clinic should come under the immediate authority of the hospital, suggests how the mental hospital can attract more personnel, and indicates how to use volunteer service for public education, besides discussing the place of government in mental health.

The volume is divided into five parts, dealing with basic considerations and program planning, the restoration of mental health including provisions for the mentally deficient, the protection of mental health, elevation of mental health, and special considerations, including world mental health. There is a useful section on visual aids and a good index. The book is intended for all who work and plan for mental health. The author's hopes should be realized, namely, that "the book will help them to achieve a perspective on the whole field—the common threads that run through it, and the interrelationship and interdependence of its parts—thereby enabling them to do more for mental health."

IRA V. HISCOCK

THERAPEUTIC USE OF ARTIFICIAL RADIOSCOPES. Edited by Paul F. Hahn. New York, John Wiley & Sons, Inc.; London, Chapman & Hall, Limited, 1956. 414 pp. $10.00.

This volume is the second of its kind by the same editor. The first one was published under the title of *A Manual of Artificial Radioscope Therapy* (New York, Academic Press, Inc., 310 pp.) in 1951 and has been well received by the radiological profession. It is noted that the editor has

obtained the assistance of an entirely different group of contributors than formerly, for this volume includes many European investigators. This seems to indicate that a broader coverage of many new advances is necessary for such a rapidly expanding field. Talents of no one individual can be expected to encompass the entire field.

This volume contains nineteen chapters. The material is well selected and organized, although some subject matter is handled at greater length than others and some minor repetitions are noted, such as definition of Roentgen Unit which appeared twice in the book. Throughout the volume, special emphasis is placed on a detailed description of dosimetry, instrumentation, procedure of administration, and radiation precaution. At the end of each chapter there is a brief but pertinent bibliography and in many chapters a summary. In a brief review of this kind only certain highlights can be emphasized.

In his "introduction to the therapeutic radiation problem" George Hevesy, a Nobel Laureate, does a magnificent job in reviewing the historical development of radioisotopes in therapeutic use and he clearly points out clinical applications, limitations, and potentialities.

The chapters on "Radiological Physics" and "Dosimetry of Therapeutic Use of Internally Administered Radioisotopes" are written in a clear, easily readable, and non-mathematical fashion. Many practical problems in radiation measurement and dosage calculations are illustrated.

The program of therapy for leukemia and polycythemia called titrated regularly spaced P 32 therapy is described in detail by E. E. Osgood, and the treatment results with a logarithmic probability analysis are presented. Treatment of chronic leukemia by intravenously administered radioactive colloidal gold is advocated by P. F. Hahn and represents an interesting and new experimental therapeutic approach, although no evidence is available to indicate that the result of this method is better than the conventional P 32 therapy.

The treatment of thyrotoxicosis by radioiodine is authoritatively presented by N. B. Myant. A very useful table containing the results obtained with radioiodine in the treatment of thyrotoxicosis from 37 reported series are compiled. The treatment of thyroid carcinoma with radioiodine is a fascinating therapeutic endeavor. Unfortunately, it has been found that only less than 10 per cent of the tumor will concentrate radioiodine and respond to the treatment.

The interstitial use of radiogold in the treatment of cancer of the prostate and the parametrial tissue in carcinoma of the cervix has aroused increasing interest. The success in the treatment depends entirely on the uniform distribution of radiocolloid in the tumor tissue and a sufficient amount of the colloid being picked up by the metastatic nodes. Unfortunately, this is not exactly the case with the colloids presently available. Further investigation along this line is worth an all-out effort.

Intracevitary application of radiocolloidal gold for palliation of effusion has become an accepted therapeutic procedure. The therapeutic dose used by J. H. Muller, originator of this type of treatment, seems to be higher

than that used by American investigators. Exploration with other pure Beta emitters in colloidal form is in progress.

Investigations in the intracavitary injections of liquid radioisotope such as sodium, bromine, and colloidal gold, or interstitial implantation of tantalum wire or gold grain for the treatment of carcinoma of the urinary bladder is reported by the Royal Marsden Hospital group of London. Plastic P 32 skin and corneal applicators also have been designed and used by this group.

Implantation therapy with artificial radioisotope is still in the developmental stage. The technical aspect in the handling of CO^{60} needles and nylon ribbon, and Au^{198} seeds is excellently presented by U. K. Henschke. The Tele-Cobalt Unit is concisely discussed by G. H. Fletcher. A chapter on "Availability and Procurement of Isotope" by D. T. Tabern enhances the practical features of the book.

In summary, this volume includes many authoritative articles representing an attempt to show a cross-section of the present effort in the States and abroad to employ artificial radioisotopes in therapy. The information is practical, up-to-date, and in many areas stimulating. All those interested in any of the numerous aspects of artificial radioisotopes, particularly radiologists and clinicians who are dealing with the treatment of benign and malignant diseases with radioisotopes, can't afford to miss this exceedingly useful book.

C. H. CHANG

FINANCING HOSPITAL CARE IN THE UNITED STATES. Volume 3. FINANCING HOSPITAL CARE FOR NONWAGE AND LOW-INCOME GROUPS. Harry Becker, Ed. New York, McGraw-Hill Book Co., Inc., 1955. xviii, 110 pp. $2.50.

This little volume contains an elementary description of the major obstacle to adequate financing of voluntary hospital care in the United States—the problem of meeting the costs of care for those who cannot pay for hospital care themselves. By summarizing materials readily available in a number of other sources, it attempts to identify the persons in this category and to describe the existing provisions and proposals for a solution to the community problem of financing their hospital care. The general recommendations of the Commission on the Financing of Hospital Care, with several dissents, complete the volume.

The Commission, an independent 34-member group of experts, was appointed in 1951 "to study the costs of providing adequate hospital services and to determine the best systems of payment for such services." The first volume of their report deals succinctly and intelligently with the intimate aspects of hospital costs. The second is concerned with the applications of voluntary prepayment devices and includes a 25-page chapter on nonwage and low income group coverage. This third volume was required because "the Commission recognized that there are many persons who, for reasons of inability to work or for reason of marginal income, are unable to pay for

hospital care . . ." And furthermore, "The inadequacy of existing methods for financing hospital care for the nonwage and low income groups is a major reason underlying the financial difficulties of America's voluntary hospital system."

The approach in this volume, and in the recommendations of the Commission, seems to rest upon the concept that the financing of hospital care is a unique and separate problem in our economy. As a result, there is implicit throughout a failure to relate hospital services to other health services. It is presumed that the consumer's health dollar, and the community's health funds as well, will be allocated on the basis of competition for these funds among the providers of service, with the hospital holding top priority. The fact that about three-fourths of consumer medical care expenditures go for other services seems to be neglected, as is the fact that the allocation of health funds should relate to the need for services of all types. In this respect, the report has echoed the existing, generally unplanned community picture with its concentration on the payment, usually inadequate, of hospital bills and the resulting costly and wasteful use of hospital resources.

The writing in this volume is choppy and many of the charts and graphs are poorly reproduced. Nevertheless, it will serve as a handy reference source for newcomers to the field. Professionals dealing with this subject may find the text adequate as a quick brush-up.

The specific recommendations of the Commission range from the generally acceptable proposition of including provision for coverage of periods of income loss due to temporary disability or unemployment in the basic prepayment premium to the highly controversial suggestion that the federal social insurance system include coverage for hospital care for "needy beneficiaries." The latter recommendation is attacked in dissenting reports from both sides of the political fence—the dissents probably adding up to the most interesting reading in the volume. Since this subject is bound to be an issue of political importance for the next several years, this discussion may even be sufficient reason for the general reader, who is concerned over community and national problems, to read, and possibly own, this third volume of the report of the Commission on the Financing of Hospital Care.

JONAS N. MULLER

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Free for the asking from Distillation Products Industries, Eastman Organic Chemicals Department, Rochester 3, N. Y., is a chart showing the overlapping infrared windows of all the Eastman Spectro Solvents. (From 2 to 16μ not a sliver has evaded overlapping.)

Movies with comment

The next step after learning to picture progress and projects on Kodachrome movies is to have us lay down a *Kodak Sonotrack Coating* along the edge of the film at the time we process it for your dealer. Then, while a *Kodascope Magnetic-Optical Sound Projector* shows the picture, appropriate remarks are spoken into the microphone. Any that seem less than appropriate when the projector plays them back with the picture have merely to be respoken. Automatically the boo-boo is wiped clean and replaced by wiser words.

This projector we have just brought out now. We hadn't thought it wise to take anybody's money for such a machine until enough organizations had used our design without trouble and had liked the way it sounds.

From Eastman Kodak Company, Cine-Kodak Equipment Sales, Rochester 4, N. Y., one may expect an honest attempt at collaboration on sensible procedures for using inexpensive sound movies in technical communication and documentation.

"I have a photo here"

You will find within the next couple of years that the mental image created by the word "photograph" will have altered. To think of a photograph as a piece of paper bearing a representation in tones of black, white, and grey will be like calling a man who flies an airplane an aviator or picturing a professor as bearded—perfectly proper but no longer general. The photographs that you file as records of work and observations and the photographs you pull out of your billfold at postprandial bull sessions will, in general, be in full color.

Here is what has been happening:

1) This year a new *Kodacolor Film* came out. It is as sensitive as the popular variety of black-and-white snapshooting film used to be not so long ago. It works equally well for daylight and clear flash without filters. It gives negatives from which can be made color prints and enlargements that you look *at*, not through.

2) There is now a *Kodak Color Print Material, Type C.* Prints made on it from Kodacolor negatives have the same color quality as used to be obtained only through vastly more involved techniques.

3) Processing chemicals for both the film and the print material are available in kits from all Kodak dealers. Quality of results tends to run commensurate with the degrees of care, zeal, and skill generated by the worker's needs or the hobbyist's self-fulfillment urge.

4) The fellow who, during the Great Depression, had some "Films Developed, Printed, and Enlarged" signs printed and placed in drug stores around town no longer operates from his kitchen. For the convenience of those who would just as soon not do it themselves, he has gone into color. To compete on both quality and price he finds it wise to own *Kodak Color Densitometers* and the like. His plant manager comes to Rochester for brush-up courses. He has met and mastered a complex technology, and he is determined to convince you that its product has it all over the monochromatic view of things.

Prices quoted are subject to change without notice.

This is one of a series of reports on the many products and services with which the Eastman Kodak Company and its divisions are . . . *serving laboratories everywhere*

Kodak
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